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THE VITAMINS

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American Chemical Society
Monograph Series

BOOK DEPARTMENT

The CHEMICAL CATALOG COMPANY, *Inc.*
ONE MADISON AVENUE, NEW YORK, U. S. A.
1922

Press of
J. J. Little & Ives Company
New York, U. S. A.

GENERAL INTRODUCTION

American Chemical Society Series of Scientific and Technologic Monographs

By arrangement with the Interallied Conference of Pure and Applied Chemistry, which met in London and Brussels in July, 1919, the American Chemical Society was to undertake the production and publication of Scientific and Technologic Monographs on chemical subjects. At the same time it was agreed that the National Research Council, in cooperation with the American Chemical Society and the American Physical Society, should undertake the production and publication of Critical Tables of Chemical and Physical Constants. The American Chemical Society and the National Research Council mutually agreed to care for these two fields of chemical development. The American Chemical Society named as Trustees, to make the necessary arrangements for the publication of the monographs, Charles L. Parsons, Secretary of the American Chemical Society, Washington, D. C.; John E. Teeple, Treasurer of the American Chemical Society, New York City; and Professor Gellert Alleman of Swarthmore College. The Trustees have arranged for the publication of the American Chemical Society series of (a) Scientific and (b) Technologic Monographs by the Chemical Catalog Company of New York City.

The Council, acting through the Committee on National Policy of the American Chemical Society, appointed the editors, named at the close of this introduction, to have charge of securing authors, and of considering critically the manuscripts prepared. The editors of each series will endeavor to select topics which are of current interest and authors who are recognized as authorities in their respective fields. The list of monographs thus far secured appears in the publisher's own announcement elsewhere in this volume.

The development of knowledge in all branches of science, and

especially in chemistry, has been so rapid during the last fifty years and the fields covered by this development have been so varied that it is difficult for any individual to keep in touch with the progress in branches of science outside his own specialty. In spite of the facilities for the examination of the literature given by Chemical Abstracts and such compendia as Beilstein's Handbuch der Organischen Chemie, Richter's Lexikon, Ostwald's Lehrbuch der Allgemeinen Chemie, Abegg's and Gmelin-Kraut's Handbuch der Anorganischen Chemie and the English and French Dictionaries of Chemistry, it often takes a great deal of time to coordinate the knowledge available upon a single topic. Consequently when men who have spent years in the study of important subjects are willing to coordinate their knowledge and present it in concise, readable form, they perform a service of the highest value to their fellow chemists.

It was with a clear recognition of the usefulness of reviews of this character that a Committee of the American Chemical Society recommended the publication of the two series of monographs under the auspices of the Society.

Two rather distinct purposes are to be served by these monographs. The first purpose, whose fulfilment will probably render to chemists in general the most important service, is to present the knowledge available upon the chosen topic in a readable form, intelligible to those whose activities may be along a wholly different line. Many chemists fail to realize how closely their investigations may be connected with other work which on the surface appears far afield from their own. These monographs will enable such men to form closer contact with the work of chemists in other lines of research. The second purpose is to promote research in the branch of science covered by the monograph, by furnishing a well digested survey of the progress already made in that field and by pointing out directions in which investigation needs to be extended. To facilitate the attainment of this purpose, it is intended to include extended references to the literature, which will enable anyone interested to follow up the subject in more detail. If the literature is so voluminous that a complete bibliography is impracticable, a critical selection will be made of those papers which are most important.

The publication of these books marks a distinct departure in the policy of the American Chemical Society inasmuch as it is a

serious attempt to found an American chemical literature without primary regard to commercial considerations. The success of the venture will depend in large part upon the measure of co-operation which can be secured in the preparation of books dealing adequately with topics of general interest; it is earnestly hoped therefore that every member of the various organizations in the chemical and allied industries will recognize the importance of the enterprise and take sufficient interest to justify it.

PREFACE

Although the chemical nature of vitamins is still unknown much of both scientific and practical importance has been learned regarding them, and the present work is designed to summarize this knowledge in as judicial a manner as possible. To those who "don't believe in vitamins because we have never seen one" or who hold that we know nothing about them chemically because no structural formula can yet be assigned, we would commend the view of Hopkins that it is easier to sympathize with the farmer who will believe in vitamins only when their price per hundredweight can be quoted in the market than with the scientific man who refrains from an endeavor to appraise their importance until they have been separated in pure condition. "To be logical he should then avert his eyes from such agents as toxins and antitoxins, not to speak of enzymes; powerful realities all of them, which though of unknown constitution by no means elude objective and quantitative study." It is hoped that the present work may do something to stimulate such quantitative research as distinguished from the merely qualitative tests which have now largely served their purpose, and at the same time may serve to present the chief facts now known in such a way as to show their true significance and avoid exaggerated impressions. To this end the non-technical reader may perhaps be advised to read the last chapter first.

The greater part of the text was written near the middle of the year 1921 but on a few points we have been able to bring our summary down to about the end of that year, thus covering the work of the decade since the term was introduced and the conception of the vitamins began to be current. A carefully selected alphabetical bibliography of about one thousand titles is included. Names and dates are so used in the text that the reader may readily locate original places of publication by turning to the bibliography, the need of foot-note references being thus avoided. It is hoped that this bibliography will suffice to put

the reader in touch with practically all of the more significant literature of the vitamins to the end of 1921. The writers will be glad to be notified of any serious omissions in case a second edition should be called for.

January 3, 1922.

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THE VITAMINS

Chapter I.

Historical Introduction to the Vitamin Theory.

Until recent years the discussion of foods and nutrition from the chemical point of view was hampered by the embarrassing fact that all attempts at prolonged feeding upon artificial mixtures containing the substances known to be necessary in nutrition had ended in failure. Nor were such feeding experiments any more successful when great care was devoted to the chemical purity of the substances fed. Whether nutritive failure resulted from the need of other substances than those known as essential, or from faulty selection or quantitative combination of the nutrients entering into the artificial food mixture remained obscure until the work of Hopkins in England and of Osborne and Mendel and McCollum and Davis in this country made it clear that a natural food supply furnishes, and normal nutrition requires, other substances in addition to proteins, fats, carbohydrates, water, and salts. With this fact now convincingly established, it is easier to see that it was foreshadowed by many earlier observations than it is to say definitely when or by whom the existence of the substances now known as vitamins was discovered. Our present "vitamin theory," or point of view in regard to this branch of food chemistry, is rather the product of development than of any isolated discovery, and much of this development antedates the introduction of the word vitamin.

Evidence from Observations upon Disease.

As early as 1720 Kramer wrote in his *Medecina Castrensis* that neither medicine nor surgery could give relief in scurvy. "But if you can get green vegetables; if you can prepare a sufficient quantity of fresh antiscorbutic juices, if you have

oranges, lemons, citrons, or their pulp and juice preserved with whey in cask, so that you can make a lemonade, or rather give to the quantity of 3 or 4 ounces of their juice in whey, you will, without other assistance, cure this dreadful evil." And Lind confirmed this doctrine by well controlled experiments upon human subjects. As surgeon of the *Salisbury* he had during one voyage twelve scurvy patients without sufficient supplies of oranges and lemons for them all. He therefore gave all the patients the best care and housing that the circumstances permitted, and to two of them he gave oranges and lemons while others received cider, cream of tartar, elixir of vitriol, or other medicaments which had been recommended by different medical writers of that day. The men receiving oranges and lemons made rapid recovery, those having cider showed improvement, while the different medicines tried were found to be without effect upon the scurvy. With these and other experiences in mind, Lind, writing in 1757, pointed out that while lemon juice retained its antiscorbutic property sufficiently well to make it useful for long voyages, there was no hope of preventing scurvy by means of dried spinach since this had lost during its preparation "something contained in the natural juices of the plant" which "no moisture whatever could replace." The idea that the antiscorbutic properties of certain foods is due to some definite (though not yet identified) thing, was again clearly stated by Budd who in 1847 ascribed the action of antiscorbutic foods "to an essential element which it is hardly too sanguine to state, will be discovered by organic chemistry or the experiments of physiologists in a not far distant future" (Hess, 1920).

In 1804 the regular issue of a ration of lemon juice was made compulsory in the British navy and thereafter scurvy was a comparatively rare disease among British sailors, whereas a few years earlier thousands of cases were reported every year. (British Committee Report, 1919, p. 58.) The relative ease with which scurvy can thus be prevented under ordinary conditions doubtless contributed to delay the rigorous search for its cause.

In the Japanese navy the disease beriberi was for a long time exceedingly prevalent. The number of cases sufficiently severe to be officially reported ranged annually from 25 to 40 per cent of the entire navy force during the years 1878 to 1882. At about this time Takaki (1885) became convinced that the diet had

some relation to the disease and succeeded in having the ration modified in several respects, the most important change being the substitution of barley for a considerable part of the polished rice which had previously been the chief article of food. Immediately following this change in the ration beriberi practically disappeared from the Japanese navy.

Eijkman in 1897 summarized the results of a large number of observations which he had made during 1890-96 upon "an illness of fowls similar to beriberi" which he was able to produce experimentally by feeding the fowls upon polished rice, and to prevent or cure by feeding an extract of the rice polishings. By means of systematic experiments he refuted the theories that beriberi might be due to the presence of pathogenic organisms in the rice, to lack of mechanical stimulation of the intestine, or to insufficiency of total food value, protein, or salts. The explanation first offered by Eijkman was to the effect that the condition is a state of intoxication brought about by the metabolism of excessive quantities of starch and that in the silverskin of the rice (and in some other foods) there is to be found a substance or substances which counteract the toxic products of the disturbed metabolism. As has been pointed out in the British Committee Report, the pharmacological bias thus given by Eijkman to the interpretation of his observations resulted in obscuring the fact that he had here adduced experimental evidence of the presence in rice polishings and some other foods, of a substance or substances playing an important part in nutrition and whose existence was previously unknown.

Later Eijkman (1906) withdrew the hypothesis of a nerve poison and stated clearly that there is present in rice polishings a substance of a different nature than protein, fat or salts which is indispensable to health and the lack of which causes "nutritional polyneuritis,"—this designation being used by Eijkman as the title of his paper of this date. Thus the existence of an antineuritic substance as something different from the known foodstuffs but essential to normal nutrition was clearly discovered by Eijkman long before the term vitamin was proposed. Eijkman's excellent pioneer work in this field does not seem to have received the attention that it deserves, either from the chemical or the physiological point of view. Even as regards the cause and cure of beriberi, the significance of his work was

not generally recognized until after several years during which much new evidence relating to the dietary origin of the disease beriberi and the substantial duplication of beriberi in the experimental polyneuritis of fowls was brought forward. Only a few of the investigations of this period can be mentioned here.

Grijns, in 1901, showed that the polyneuritis of fowls, produced by feeding polished rice, could be prevented by adding the native bean, katjang idjo (*Phaseolus radiatus*), to the polished rice diet. Hulshoff-Pol (1902) then tested this bean and found it effective both as a preventive and curative agent in human beriberi. He emphasized: (1) that a diet consisting too largely of highly milled rice will produce beriberi, (2) that the disease can be prevented by the addition of beans to the diet, (3) that beriberi must be due to some deficiency in the diet since if the rice were toxic the disease could hardly be prevented simply by the addition of the beans. He then made a decoction of the beans which he showed to contain the substance needed for the cure of beriberi by the successful treatment of 18 patients. As a further step he purified the decoction by precipitation with basic lead acetate and subsequent removal of the lead. With this purified preparation he cured four cases of human beriberi, and both cured and prevented the polyneuritis of fowls. Plainly these experiments afforded very strong evidence that the polyneuritis of fowls induced by feeding polished rice is essentially the same condition as beriberi and that both are due to the lack of a definite substance, which occurs in rice polishings, beans, and some other foods.

Chamberlain and Vedder, of the United States Army medical commission for the study of tropical diseases in the Philippines, made considerable progress in the study of the chemical nature of the substance which prevents and cures beriberi in man and experimental polyneuritis in fowls. They found that this antineuritic substance was soluble in water and in alcohol, dialyzable, moderately thermostable, more readily destroyed in alkaline than in neutral or acid solutions, and that it was neither sugar, a salt, an alkaloid, nor any of the organic phosphorus compounds nor amino acids which were available for their experiments. They believed that it was an organic base but not an alkaloid.

The work looking toward chemical identification of the active

substance thus begun by Hulshoff-Pol and by Chamberlain and Vedder was then taken up by Funk, who in a paper published in December 1911, first claimed to have isolated the active substance in an approximately pure state. He gave to it the name *beriberi vitamine.* The name *vitamine* thus introduced by Funk was obviously designed to indicate that the substance in question was an amine essential to life or bearing special relationship to vitality. He believed this substance to be a combination of nicotinic acid with a pyrimidine base, and predicted that other substances would be found to bear the same relation to other "deficiency diseases" which this "beriberi vitamine" bears to beriberi and experimental polyneuritis.

At practically the same time that Funk put forward this suggestion, Holst and Frölich of Christiania published (1912) the results of several years of systematic work upon experimental scurvy. Because of its importance to the Norwegian fisherman and merchant marine, they had been interested in the study of ship beriberi and had felt that the application of laboratory experiments to human therapeutics and prophylaxis would rest upon a more satisfactory basis if they could produce the disease experimentally in mammals in preference to fowls or pigeons. They found, however, that the guinea-pig when kept upon a diet of polished rice developed the symptoms of scurvy instead of beriberi, and that scurvy also developed when whole grains, such as would ensure protection against beriberi, were fed. From this they proceeded to a thorough study of experimental scurvy in the guinea-pig, the results of which (to be described in Chapter III) indicated clearly that scurvy is due to the deficiency in the diet of a substance soluble in water and easily destroyed by heating, but more stable in acid than in neutral or alkaline medium.

Shortly after this, Osborne and Mendel (1913) found that their experimental animals became subject to a characteristic eye disease when kept upon diets devoid of fat, while the simple addition of butter fat to the diet sufficed either to prevent or to cure the disease. Further experiments showed that cod liver oil shared this property with butter fat, and that beef fat showed it in lesser degree, while it was not shown under like conditions by certain other fats such as lard or cottonseed oil. As this eye disease involves an infection it is not so purely a "deficiency

disease" as scurvy and beriberi now appear to be, yet this distinction does not seem to us to be particularly significant since the susceptibility to the eye trouble induced by a deficiency in the diet is the chief determining cause of the disease.

Thus observations upon disease led to the conception of three or more substances of the vitamin type, two soluble in water and needed for the prevention of beriberi and scurvy respectively,

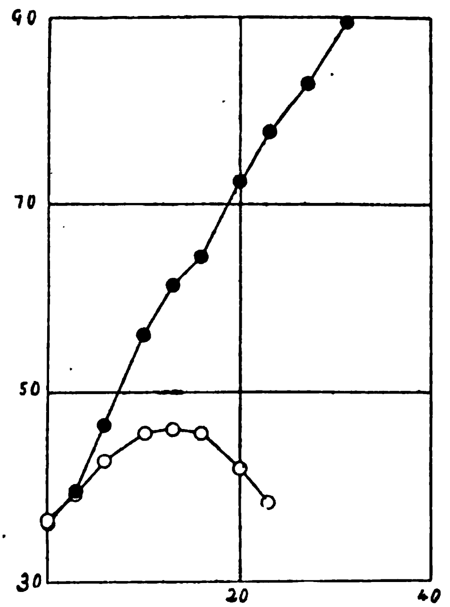


FIG. 1.—Growth curves of rats with and without the vitamins furnished by small amounts of milk. The lower curve shows the average weight of six rats receiving a diet of purified foodstuffs; the upper curve that of six similar rats receiving the same diet with the addition of small amounts of milk. Abscissæ—time in days; ordinates—average weight in grams. (Courtesy of Professor Hopkins and the Medical Research Committee of Great Britain.)

and one soluble in fats and so essential to a normal condition of healthy resistance that its absence from the diet results in susceptibility to the characteristic eye disease variously referred to as ophthalmia, xerophthalmia, conjunctivitis or keratomalacia.

While in this way the conception of the vitamins has arisen largely in connection, and is intimately associated, with that of the deficiency diseases, yet from our present standpoint an even greater interest attaches to them as chemical substances occurring

in natural food materials and having important functions in the normal processes of nutrition.

Evidence from Experiments upon Normal Nutrition.

In 1881, Lunin in the course of a report upon investigations of the significance of certain inorganic substances in animal nutrition remarked that since mice can be successfully nourished under laboratory conditions upon milk but not upon purified

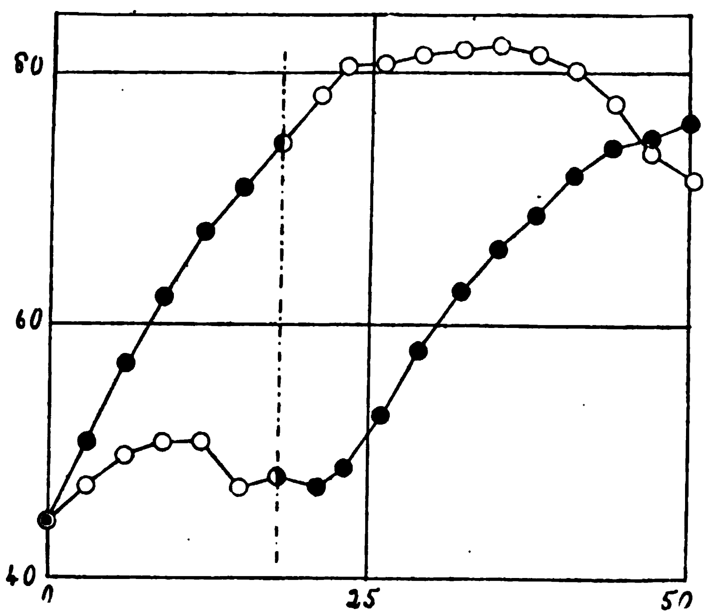


FIG. 2.—Growth curves of rats with and without small amounts of milk as source of vitamins. The lower curve up to the eighteenth day represents rats on purified food; the upper curve similar rats having milk each day in addition to this food. On the eighteenth day marked by the vertical dotted line the milk was transferred from one set to the other. Abscissæ—time in days; ordinates—average weight in grams. (Courtesy of Professor Hopkins and the Medical Research Committee of Great Britain.)

proteins, fats, carbohydrates, salts and water, it follows that milk must contain other substances indispensable for nutrition. This statement was given wide circulation in Bunge's *Textbook of Physiological and Pathological Chemistry*, but never became influential, probably because the evidence upon which it was based was not sufficiently convincing.

To Hopkins is due, in our opinion, the honor of having first made clear that natural food contains and normal nutrition requires some other substance or substances beside proteins, fats, carbohydrates and mineral matters. As early as 1906 he had determined and reported that "no animal can live upon a mixture of pure protein, fat and carbohydrate and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or other animals and these contain countless substances other than the proteins, carbohydrates and fats." In his experiments, using rats as subjects, Hopkins found that the addition of small amounts of milk to diets otherwise composed of purified foodstuffs resulted in growth (Figs. 1 and 2), and that this was due to an alcohol-soluble organic substance or substances in the milk and not to any of its known constituents. Certain vegetables had the same property in lesser degree than milk. Osborne and Mendel demonstrated that a similar growth-promoting property was possessed by their "protein-free milk," a powder prepared by removing the fat, casein and albumin from cows' milk and evaporating the filtrate to dryness. A little later it was found both by McCollum and Davis and by Osborne and Mendel that the fat of milk also possesses a growth-promoting property which is not an attribute of the triglycerides themselves but rather of a fat-soluble substance. Both the water-soluble and the fat-soluble growth-promoting substances are fairly soluble in alcohol which accounts for the fact that Hopkins' alcoholic extract of dry milk contained both of these essentials and supplied all that was needed for the growth of rats when added to his mixture of previously recognized foodstuffs. The theory soon gained currency and is still generally held that the water-soluble and fat-soluble substances essential to growth are identical with the water-soluble and fat-soluble "vitamins" which prevent beriberi and ophthalmia respectively. The addition of these two vitamins to a diet otherwise consisting of properly selected isolated foodstuffs appears to provide all that is needed for the growth of rats. The normal growth of babies and of young guinea-pigs requires, however, the feeding of sufficient amounts of the antiscorbutic vitamin as well.

Hence normal nutrition, at least in the human and some other

species, demands adequate supplies of all three of what are believed to be the same vitamins which are essential to the prevention of the three "deficiency diseases" of scurvy, beriberi, and ophthalmia.

Terminology.

The names which seem best to express the distinctive properties of the three vitamins as at present known are perhaps "antineuritic," "antiscorbutic," "antiophthalmic" (or "anticonjunctivitic") vitamin respectively. From the standpoint of food chemistry and normal nutrition, however, it seems a somewhat round-about procedure that a substance having an important rôle in normal processes should be named according to the abnormal condition which arises when it is absent. Moreover the term "vitamine" has been criticised both because it implies that these substances are amines, which is not proven in any case and certainly not probable in all, and because the choice of "vita" as a designation is thought by some to carry an exaggerated implication of unique responsibility for life and vitality whereas other substances such as tryptophane are no less essential. But if "vita" seems to claim too much, the designation "accessory" suggested by Hopkins is certainly too modest, since an accessory substance would ordinarily be judged to be dispensable whereas the indispensability of these substances is one of their most marked characteristics.

In order to avoid these difficulties McCollum suggested that until such time as chemical names can properly be assigned to them, these substances be known by alphabetical designations qualified only by such statement of their solubilities as may seem helpful. In his earlier rations of supposedly purified foodstuffs McCollum unwittingly furnished water-soluble vitamin in the lactose fed. Hence he at first concluded that the fat-soluble substance of butter, egg-fat, etc., was the only unidentified substance essential to normal nutrition. Thus this became, in his alphabetical terminology, "fat-soluble A," and the water-soluble substance although really discovered much earlier was designated as "water-soluble B." For a time McCollum actively opposed the view that a third (antiscorbutic) substance was also necessary, but as the evidence of its existence became indubitable this latter was included in the alphabetical terminology as

"water-soluble C." The lack of harmony between historical and alphabetical sequence and the somewhat exaggerated emphasis upon apparent solubility are unfortunate features of this system of terminology.

The designation of these substances as "food hormones," used in some of their publications by Osborne and Mendel and favored by Lusk in the third edition of his *Science of Nutrition,* would avoid the objectionable features of the term vitamin and would emphasize the conspicuous characteristics of these substances; but might perhaps be open to objection as suggesting solely regulatory to the exclusion of structural functions, which would again be introducing a connotation which might or might not be substantiated by future research.

Quite recently Drummond (1920) has suggested that the designations now most common, those of Funk and of McCollum, be combined and simplified both for convenience and to free them from questionable implications as follows: That the alphabetical designations now familiar be retained but without the antecedent statements of solubility; and that the original designation of Funk be retained but the final "e" be dropped, so that the resulting word Vitamin shall carry no implication as to the chemical constitution of the substance. If this suggestion is adopted the three substances now recognized as belonging to this group may be designated as Vitamin A, B, and C respectively, and any others whose existence may be demonstrated before they are chemically identified, can be labelled alphabetically in chronological order. In view of the wide currency which the term vitamin has attained, this suggestion seems as practical as any and will be employed when convenient in the present work.

It is also to be kept in mind that one or more of the present designations "A," "B," and "C" may be found to cover more than one substance. Hence we consider it desirable to make sufficient use of the physiological designations to ensure that we keep in mind in all discussions of these substances the experimental basis for our belief in their actual and independent existence, and the fact that present evidence does not exclude the possible existence of other substances belonging to the same general category. Especially important is it in our judgment to avoid habits of thought or expression which might prejudice the

interpretation of experiments now in progress or yet to be made to determine, for instance, whether the antineuritic and the water-soluble growth-promoting substances are the same, or whether the antiscorbutic "vitamin" is capable of existence in a less active, more stable form as well as in the form of the heat-labile substance "water-soluble C."

As the vitamin theory of the present day had its origin in the study of the antineuritic substance and the fundamental conception of the vitamins has so largely grown up around the properties of the substance to which the name was first given, we shall, in what follows, discuss first the antineuritic vitamin and the supposedly identical "water-soluble B" and then differentiate the other vitamins from this.

Chapter II.

The Antineuritic Vitamin (and, or) Vitamin B.

We have seen that Eijkman, in his papers published from 1897 to 1906, clearly set forth his discovery that a diet of polished rice produces in fowls a condition of nutritional polyneuritis which appears to be essentially identical with typical beriberi in man, and that this disease is due to the lack of a substance essential to normal nutrition, which substance exists in the rice polishings (i.e., in the outer layers and embryo of the rice kernel) as well as in other natural foods. After the publication of the first of these papers, Grijns (1901) took up the subject, confirmed Eijkman's earlier work and extended it to show that the antineuritic or protective substance which Eijkman had demonstrated to occur in rice, occurs also in legumes and in this case not wholly in the outer layers of the seed. It is interesting to note that at this time he recorded the further fact that the antineuritic substance seemed to disappear when the seeds (*Phaseolus radiatus*) were germinated, a circumstance which may have special significance in connection with the simultaneous development of the antiscorbutic property (Chapter III).

Failure to isolate the antineuritic substance from rice polishings led Grijns to determine whether the heating of unpolished rice (or of the phaseolus seeds) destroyed the protective power. Of four fowls fed unpolished rice which had been heated at 120° C, two died of polyneuritis after five or six months, while two remained well at the end of eleven months. On polished rice fowls may be expected to develop polyneuritis in about three weeks, while with rice unpolished and unheated there should be entire freedom from the disease. Hence Grijns concluded that heating at 120° destroyed much but not all of the antineuritic substance of rice. Similarly heating the beans (phaseolus) for one hour at 120° destroyed their antineuritic property to such

an extent that four fowls, fed such quantities of the beans as would ordinarily protect them, all died of polyneuritis in from 33 to 37 days. Grijns also observed that fowls fed raw meat were protected, while those fed sterilized meat were not.

Eijkman in 1906 described experiments designed to throw light upon the chemical nature of the antineuritic substance. He found that it was soluble in water, was dialyzable, and was not readily precipitated from water solution by alcohol. In Eijkman's experiments the antineuritic substance appeared to be entirely destroyed when unpolished rice was heated in an autoclave for two hours at 125°, but only partially destroyed when the temperature of heating was 115°. Similar results were obtained in experiments with other grains. Eijkman also showed that fowls rendered polyneuritic by feeding with grain which had been heated in the autoclave could be cured by giving water extracts of raw grain but not by phosphorus compounds prepared from such extracts—a result of considerable importance at that date since under the teaching of Schaumann there was a tendency to ascribe the antineuritic property to some organic phosphorus compound. As further evidence on this point Eijkman showed in 1911 that an extract of rice polishings containing only the slightest traces of phosphorus yet had marked curative effects in polyneuritis. He also showed that the curative substances could be administered either by the mouth or by injection.

Simultaneous with much of this work by Eijkman were the experiments of Hopkins (1906, 1912) and those of Osborne and Mendel (1911). The former showed that milk and some at least of the vegetables contained an organic substance or substances soluble in water and alcohol which induced growth in young animals fed upon mixtures of purified foodstuffs whereas when these foodstuffs were well purified and fed without accessory substances growth always failed. Osborne and Mendel further showed that the water solution remaining after removal of fat, casein and lactalbumin from milk (the so-called protein-free milk) was much more efficient in inducing growth than was a corresponding mixture of lactose and pure salts or milk ash, thus implying the presence of some water-soluble organic growth-promoting substance. In later papers they have fully confirmed this early inference and have contributed much to the develop-

ment of our knowledge of the distribution of such a vitamin among foods and its significance in normal nutrition, particularly in connection with growth.

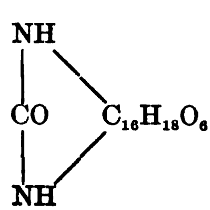
It was, however, principally in connection with the study of beriberi and experimental polyneuritis that the vitamin conception was actively developed at the beginning of the second decade of this century.

Takaki's conquest of beriberi in the Japanese navy by a change of ration did not carry conviction to many at the time because the only explanation which he had to offer, namely that the new ration was richer in protein than the old, was seen to be inadequate, and the work of Eijkman and of Grijns did not at first command much attention because many were slow to accept the view that the experimental polyneuritis of fowls was the same disease as human beriberi. Between 1900 and 1910, however, there was accumulated indubitable evidence in support of the view that beriberi is essentially a nutritional disease and may be prevented by the consumption of adequate amounts of any of the foods containing the unknown antineuritic substance.

Chamberlain, Vedder and their associates of the United States Army medical commission for the study of tropical diseases in the Philippines were able to report in 1910 the rapid eradication of beriberi from the native troops known as Philippine Scouts, and promptly began a systematic investigation looking toward the chemical identification of the antineuritic substance. As has been mentioned briefly in the preceding chapter, they found that the neuritis-preventing substance is soluble in water and cold alcohol, that it is not any one of a number of salts and organic phosphorus compounds which they tested, that it is capable of dialyzing readily through an ordinary parchment membrane and is therefore a crystalloid rather than a colloid, that it is rather readily adsorbed by bone black, can be decomposed by heat, is not soluble in ether nor is it choline or a lipoid of the lecithin group, that it is neither arginine, histidine, asparagine nor any of the other amino acids which they tried. They suggested that though not identical with any of these nitrogen compounds the antineuritic substance might prove to be a nitrogenous base but not an alkaloid. Simultaneously with this work, Fraser and Stanton (1911) had shown that the antineuritic substance was soluble in both alcohol and water,

stable to heat in acid solution, and much more readily destroyed when the solution was alkaline. In attempts to isolate the protective substance they had obtained an active filtrate by extracting rice polishings with 0.3 per cent hydrochloric acid and precipitating phytin by adding one and one half volumes of 95 per cent alcohol. Attempts at further purification of the active substance contained in this filtrate proved unsuccessful.

Cooper and Funk (1911) reported that dried, pressed yeast hydrolyzed for 24 hours with 20 per cent sulfuric acid still retained its curative properties. They also confirmed the results of Fraser and Stanton concerning the antineuritic action of the alcoholic extract of rice polishings and carried the process further by finding that the active substance was completely precipitated from a water solution of the extract by means of phosphotungstic acid and that on decomposing the precipitate with barium hydroxide an active substance devoid of phosphorus and free from carbohydrate and protein was obtained. Following this, Funk (1911) announced the isolation from rice polishings of a crystalline nitrogenous compound which he held to be the curative substance. To this product he at first assigned the formula $C_{17}H_{19}O_4N(HNO_3)$. Shortly afterward (1912a) he corrected his previous statement that the curative substance, which he provisionally named *beriberi vitamine* (1912c), was isolated in the form of a nitrate, later evidence indicating that it was a free base probably belonging to the pyrimidine group analogous to uracil and thymine and possibly a constituent of nucleic acid. A formula suggested at this time was



While later work has hardly supported such definite conclusions as to the chemical nature of the antineuritic substance, yet considerable interest attaches to the experimental evidence which led to the introduction of the term vitamin and helped to crystallize the conception for which the term stands.

The method of extraction first adopted by Funk (1911) was a modification of that used by Fraser and Stanton, based on the customary procedure for isolating simple natural bases. On account of the small amount of curative substance present 54 kilograms of rice polishings were used. This was extracted in separate portions of 1500 grams each with 4 liters of alcohol containing gaseous hydrochloric acid to the extent of from two to five per cent, the extraction being conducted in a shaking machine and the liquid afterwards filtered on a Buchner funnel, the residue pressed out in an hydraulic press and the liquid added to the original filtrate. On evaporation in vacuo at 38° C. a fat-like residue was obtained which melted at 50°. This was heated with water on a water bath, and filtered at 38°-40°. The filtrate formed two layers which were separated and the aqueous layer extracted three times with ether to remove fatty substances. The resulting aqueous solution, which is described as containing no proteins and showing no reaction for amino acids when subjected to the Millon, glyoxylic acid, bromin, xanthoproteic, diazo and diacetyl tests, was found to be effective in curing polyneuritic pigeons when doses corresponding to 20 grams of the original rice were given. On adding to such an aqueous extract sulfuric acid to five per cent concentration followed by phosphotungstic acid the active substance came down in the precipitate while the filtrate, although containing nitrogen, proved inactive. The precipitate was washed with five per cent sulfuric acid, ground with barium hydroxide in a mortar, water added, and the mixture shaken for three hours, then filtered and the precipitated barium phosphotungstate washed with water. The filtrate, after removal of ammonia by aeration and excess barium by precipitation with sulfuric acid and filtration, was neutralized with hydrochloric acid and evaporated in vacuo at room temperature. The alcoholic extract of this residue in doses equivalent to 40 grams of the rice polishings was an effective cure for polyneuritic pigeons. It contained no proteins, phosphorus, or carbohydrates.

On treating the above extract with alcoholic mercuric chloride and allowing the solution to stand in the cold for some time a crystalline precipitate (I) was obtained which was soluble in water and recrystallized from the water solution in needle-like crystals consisting largely of choline but containing some of the active substance. At this point the active substance was also found in the aqueous filtrate after recrystallization (II) and in the alcoholic filtrate from the mercuric chloride precipitate (III). These filtrates were treated separately as follows: The mercury was removed from the aqueous filtrate (II) by hydrogen sulfide, the filtrate evaporated, the residue taken up by alcohol, the choline removed by precipitation with platinum chloride and filtration, and the filtrate finally treated with phosphotungstic acid. A crystalline precipitate was obtained which showed activity on decomposition of the phosphotungstate with barium hydroxide and carbon dioxide. The alcoholic filtrate (III) was evaporated, the residue taken up in water, the mercury removed by hydrogen sulfide and the filtrate freed from chloride by silver sulfate, the silver removed by hydrogen sulfide, and the sulfuric acid by barium hydroxide. The filtrate thus purified was then acidified with nitric acid and treated with silver nitrate and barium hydroxide according to the methods used in isolating histidine and like bases as described by Barger (1914), the silver nitrate being added until a drop of the solution gave a brown color on the addition of barium hydroxide and the barium hydroxide subsequently added until a drop of the solution no longer gave a white precipitate with ammoniacal silver nitrate. The precipitate thus obtained was washed well with nitric acid, decomposed with hydrogen sulfide, freed from the last traces of barium hydroxide with very dilute sulfuric acid and concentrated in vacuo. On evaporating with alcohol in a desiccator the active extract thus prepared, a small yield (0.4 gram from 50 kilograms of the rice polishings) of microscopic needle-like crys-

tals was obtained which in doses of about .02 gram effected a rapid cure in polyneuritic pigeons. These crystals melted at 233° C, were difficultly soluble in cold water and alcohol, more soluble in hot water and were free from chlorine, ash, and sulfuric acid. A single analysis of the substance gave the percentage composition: Carbon, 55.63; Hydrogen, 5.29; Nitrogen, 7.68 per cent, figures which were thought to correspond best to the formula $C_{17}H_{20}O_7N_2$ or $C_{17}H_{19}O_4N(HNO_3)$ as first suggested.

The following year Funk (1912a) reported the isolation of the same or similar substances from yeast, milk, ox brain and lime juice. The yeast was treated in two different ways. In the first, the alcoholic extract was evaporated and the residue hydrolyzed with sulfuric acid after which the solution was treated as in the case of the rice polishings by precipitation first with phosphotungstic acid and then with silver nitrate and barium hydroxide. In the second method the alcoholic extract after evaporation was extracted with water and then precipitated with silver nitrate and barium hydroxide. This method proved unsatisfactory as the bulk of the vitamin remained in the filtrate and could not be precipitated except after hydrolysis. This was thought to indicate that the vitamin is present in yeast in a combined form.

While later work appears to show that the crystals obtained by Funk from rice polishings, and melting at 233°, were chiefly nicotinic acid, he holds that the product which he prepared from yeast was different and represents, he believes, the vitamin itself. The technique of the method of preparation from yeast as described by him is as follows:

"Seventy-five kilograms of air-dried and pulverized yeast were extracted in portions of 2½ kilograms each with four liters of alcohol on the shaking machine for two hours. The yeast was then filtered off and the filtrate evaporated in vacuo. The residue, corresponding to 12½ kilograms of yeast in each case, was hydrolysed with one liter of 10 per cent sulfuric acid for five hours. The fatty acids were filtered off and the filtrate diluted with water to obtain a 5 per cent solution of sulfuric acid and precipitated with phosphotungstic acid. In this way 927 grams of dry phosphotungstate were obtained from 75 kilograms of yeast. The precipitate was decomposed with 2500 grams of baryta in the usual way. In the filtrate the excess of baryta was eliminated carefully with sulfuric acid and the filtrate after neutralization with nitric acid was evaporated in vacuo to a volume of one liter. To the liquid a silver nitrate solution was added until a drop gave with a cold solution of baryta a brown precipitate of silver oxide. A bulky precipitate consisting of purine bases separated, which was filtered and to the filtrate pulverized baryta was added until a drop of the solution gave with silver nitrate and ammonia only a trace of a white precipitate. The precipitate thus formed was filtered off, thoroughly washed with water and decomposed with hydrogen sulfide. From the filtrate the last traces of baryta were eliminated carefully with

a very dilute solution of sulfuric acid. Alcohol was added and the solution evaporated in a vacuum desiccator, 0.6 gram of a crystalline substance being obtained which was recrystallized from hot dilute alcohol. On cooling 0.45 gram of colorless needles separated which after drying melted at 233°, the same melting point as that of the substance from rice polishings. The substance was precipitated by mercuric acetate but not by mercuric nitrate and sulfate. When its solution was boiled with cupric oxide no copper salt was formed. The substance gives no precipitate with nitron and cannot, therefore, be a nitrate."

In June 1912 Schaumann (1912a) described the preparation of a crystallizable active base similar to that of Funk and proposed the theory that this substance functioned as an activator in the body, helping to restore to normal the degenerated nerve tissues. His work contributed little so far as methods of isolation are concerned as he used the mercuric chloride precipitation which as in the case of Funk's early work left the active substance scattered in three or four fractions.

Edie, Evans, Moore, Simpson, and Webster (1912) after considerable experimentation finally adopted the following method for the preparation of the active constituent from yeast:

Commercial fresh pressed yeast was extracted in the cold with successive quantities of methyl alcohol, filtered through thick cloth and the filtrate, after being freed from alcohol by evaporation at room temperature with the aid of an electric fan, was mixed with sufficient plaster of Paris to make it set. The matrix after standing over night was ground to a powder and extracted in a shaking machine with successive small portions of methyl alcohol made slightly acid with hydrochloric acid. The extract freed from alcohol as before was precipitated with basic lead acetate, the precipitate discarded and the filtrate freed from lead by hydrogen sulfide and then concentrated to a sirup in vacuo at 38° C. This sirup was treated with absolute alcohol, the sticky hygroscopic yellow precipitate (creatinine, etc.) filtered off, the filtrate again freed from alcohol and then precipitated with silver nitrate and barium hydroxide. This precipitate was decomposed with hydrogen sulfide, filtered and the filtrate after being freed from hydrogen sulfide evaporated to dryness in vacuo at 38°. There was obtained in this way a small quantity of a brown, sticky, hygroscopic mass, easily soluble in cold water and intensely active.

"A dose of 0.006 gram administered to a bird with severe convulsions and lameness, relieved the convulsions in four hours; the bird was flying strongly in 20 hours, and the lameness disappeared in 48 hours. Two further doses of 0.003 gram were given on the third and eighth days; the bird appeared normal, and gained weight on polished rice diet, but died on the fifteenth day without return of lameness or convulsions." They report that other results were equally favorable and that the doses of three milligrams corresponded to 15 grams of yeast.

Further work showed the sticky mass above mentioned to be insoluble in ether or acetone but to be soluble in alcohol, crystallizing in feathery crystals which on analysis gave results corresponding to a formula $C_7H_{17}N_2O_6$ or $C_7H_{16}NO_3$ (HNO_3). Assuming the presence of the trimethylamine group the formula was further written as $(CH_3)_3N.C_4H_7O_3.HNO_3$. For this crystalline substance the name *toruline* was proposed.

In July, 1912, Suzuki, Shimamura and Odake published the results of a detailed investigation much of which they describe as having antedated Funk's experiments. These Japanese investigators, working with rice polishings and preparing therefrom a substance of high antineuritic potency, gave this substance the name *oryzanine*. Since according to Suzuki, Shimamura, and Odake their paper published in 1912 represented the results of four years of chemical investigation of the antineuritic substance of rice bran, apparently quite independent of other work which was in progress at the time, and since the methods employed differed considerably from those already discussed, it seems of interest to give the chief features of their method and results as follows:

Three hundred grams of fat-free rice bran were boiled with one liter of alcohol (85 to 90 per cent) under a reflux condenser, filtered hot, the residue boiled one hour with one-half liter of alcohol, then drained, and this operation repeated four times. The entire alcoholic extract was evaporated under reduced pressure until the alcohol was completely removed. The remaining thick brown syrup was then shaken with ether, in order to extract the fats, organic acids, lecithin and other impurities, and once more evaporated at low temperature. There was thus obtained a fairly strongly acid-reacting brown syrup which was called the "alcoholic extract." The amount obtained was 30 grams or ten per cent of the weight of the original bran.

The alcoholic extract was diluted with water to 100 cubic centimeters, sulfuric acid added to 3 per cent concentration, and the liquid treated with a 30 per cent phosphotungstic acid solution to complete precipitation, avoiding an excess of the reagent. After a few hours the precipitate was filtered off, washed once with 3 per cent sulfuric acid, placed in a mortar, some water added, and the mixture triturated with an excess of barium hydroxide until the thick paste reacted strongly alkaline, or the precipitate was dissolved in acetone and water and enough barium hydroxide added to give a strongly alkaline reaction. After some time this was filtered and the residue treated three times in the same manner. The entire filtrate was carefully freed from barium by sulfuric acid, and evaporated at low temperature under reduced pressure. There remained a slightly acid, light brown syrup, which on drying over sulfuric acid formed a resinous mass to which the name Crude Oryzanine I was given. The yield was 0.4 per cent of the original bran (1.2 grams from 300 grams bran).

Crude Oryzanine I proved to be ten times as active as the alcoholic extract. From 0.03 to 0.04 gram dissolved in a little water and administered either by mouth or subcutaneously cured polyneuritic pigeons in a day while half the dose prolonged the life of the pigeon but did not effect a complete cure. It was readily soluble in water and dilute alcohol forming a slightly acid solution which gave no biuret reaction, a deep red color with Millon's reagent, and a flocculent precipitate with phosphotungstic or phosphomolybdic acid in acid solution, and in concentrated solution was precipitated by lead acetate and ammonia and partially precipitated by mercuric chloride, acetate, or nitrate or by tannic acid. On hydrolysis by dilute acid two acids described as α- and β-acids were obtained as well as nicotinic acid, and glucose. To the α-acid was ascribed the formula C[illegible]H[illegible]NO[illegible] and to the β-acid, C[illegible]H[illegible]N[illegible]O[illegible].

For further purification 4 grams Crude Oryzanine I were dissolved in 100 cubic centimeters water and treated with 20 per cent aqueous tannic acid until a slight turbidity appeared. The whitish brown flocculent precipitate was filtered out, and quickly washed with a little 1 per cent tannin solution, avoiding excess. The precipitate was then dried on a porous plate and afterward dissolved in water and acetone, decomposed with barium hydroxide, the barium removed by sulfuric acid and the resulting filtrate evaporated in vacuo, yielding a small amount of light brown syrup, "Crude Oryzanine II." The yield was only about 6 per cent of the weight of Crude Oryzanine I taken for purification. The antineuritic activity of Crude Oryzanine II was about three times that of Crude Oryzanine I, 0.01 gram of the former proving sufficient to cure a polyneuritic pigeon or to protect a healthy one.

Several modifications of this method are described as yielding similar products.

Suzuki, Shimamura and Odake state that after repeated trials they succeeded in obtaining crystals of oryzanine picrate. They were of the opinion that Crude Oryzanine II still contained some nicotinic acid which also formed a picrate hard to separate from the oryzanine picrate. By adding small amounts of the picric acid and triturating in the cold, the oryzanine picrate appeared as a yellow brown, flocculent precipitate becoming crystalline on standing in the cold while the nicotinic acid remained in solution. For further purification, the oryzanine picrate was dissolved in a little cold acetone and slowly concentrated over sulfuric acid. In this way they obtained the picrate as yellowish brown, microscopic needles, generally in clusters. It was insoluble in ether or petroleum ether, rather difficultly soluble in cold water, and readily soluble in hot water or alcohol. The yield of picrate was unfortunately so small that Suzuki, Shimamura and Odake were unable to give an exact description or to obtain the free oryzanine. It was not settled whether pure oryzanine yields on hydrolysis the α- and β- acids, and other products mentioned above.

Pigeons required for protection 3 grams bran daily, or 0.3 gram alcoholic extract, or 0.03 gram Crude Oryzanine I, or 0.01 gram Crude Oryzanine II, or about 0.005 gram "Pure Oryzanine." This would imply that the purification process had concentrated the active substance at least 500-fold. If the final substance were in fact pure, then the concentration of this substance in the original rice bran was presumably about 0.2 per cent, or in the whole rice grain perhaps about 0.05 per cent. If, however, their final product was still a mixture, then the percentage of

the actual antineuritic substance in the rice kernel must be smaller than this estimate would indicate.

Suzuki and his coworkers also attempted by feeding experiments to determine whether oryzanine plays as important a rôle with other species as with pigeons. It proved indispensable for fowls, mice and dogs. Mice generally died in ten to fifteen days when fed exclusively on polished rice, but remained sound and normal a longer time if given the alcoholic extract of bran, or Crude Oryzanine. Dogs fed on cooked rice and the boiled-out residue of horse flesh were normal at the start, but two or three weeks later the appetite began to weaken and after five to seven weeks the animals had undergone a great loss in weight. But it was found that 3 or 4 grams of alcoholic extract, or 0.3 to 0.4 gram of Crude Oryzanine I, would cure in a few days a dog which had appeared about to die. The appetite quickly came back and the body weight increased. If the doses of oryzanine were stopped the dog became sick again. In a full grown dog this change was brought about four times in seven months.

These experiments showed that in addition to protein, fat, carbohydrate, and salts the animals require oryzanine. To study this hypothesis further, pigeons and mice were fed a mixture of individual isolated food substances. Two pigeons were fed with potato starch, peptone, lecithin, phytin, and salts; two others received the same food plus 0.03 gram Crude Oryzanine I. The difference between the two groups was astonishing. The first two pigeons underwent in 10 to 15 days a great loss in weight, while the other two not only remained sound but showed a gain in weight. Instead of peptone, Suzuki, Shimamura and Odake also used casein, egg albumin, and bran protein (the latter was extracted from the bran with dilute alkali and precipitated with acetic acid). The result was always the same. Other pigeons were fed a protein-free diet, on which they naturally could not live long. However, the ones that had oryzanine lived three times as long as the others. The necessity of oryzanine was thought to be thus established, but its rôle in the animal organism to be little understood.

We have given considerable space to this paper by Suzuki, Shimamura and Odake because it appears to represent a large amount of careful work which would seem to be deserving of fuller recognition than it has received, both in justice to its

authors and for the guidance of future investigators. We are, however, at a loss to explain why if they had succeeded so well in preparing a small amount of pure oryzanine picrate in 1912, they have not since prepared it in larger quantity and completed its chemical identification.

Vedder and Williams (1913) reported observations which raised the question whether rice polishings contain other antineuritic substances than the vitamin as apparently isolated by Funk or whether the one substance is only partially separated by his method. They stated that before any of Funk's papers had reached them in the Philippines they had tried precipitating alcoholic extracts of rice polishings with phosphotungstic acid and had decomposed the phosphotungstates with barium hydroxide but had not succeeded in obtaining any protective action with the resulting material. In comparing methods they found that while Funk had added 2.5 per cent hydrochloric acid to the alcohol used for the extraction and had tested the various substances for activity by curative experiments, they had omitted the acid and tested for activity by preventive methods. On modifying their procedure to conform with Funk's, by hydrolyzing the alcoholic extract with five per cent sulfuric acid they were able to confirm his results in curative experiments. It was observed that the extract prepared with a preliminary acid hydrolysis according to Funk differed from their original unhydrolyzed extract in that the former was poisonous and promptly cured paralytic symptoms, while the latter was not poisonous and only slowly curative. They estimated that from 96 to 98 per cent of the curative substance is lost in Funk's method of decomposing the phosphotungstate precipitate with barium hydroxide. In order to reduce this destruction of the vitamin, they modified the method by extracting the phosphotungstate precipitate repeatedly by prolonged shaking with 50 per cent alcohol and then removing the phosphotungstic acid from the alcoholic solution by means of barium hydroxide. On testing the various fractions they found that polyneuritis of fowls was prevented not only by the fraction containing Funk's base but also by the filtrate from it, and even by the "purine fraction" precipitated by silver nitrate from neutral solution if this fraction were fed in sufficient quantity.

It was considered of significance that of these three groups

of substances which conferred protection only one was promptly curative, namely, the fraction containing Funk's base. They concluded that their extract contained (1) "A substance (Funk's base?) which used in sufficient doses will both protect fowls from developing polyneuritis and promptly cure fowls that have already developed the disease, and (2) two other groups of substances which will protect fowls from developing polyneuritis but which are incapable of promptly curing fowls already suffering from the disease. The latter groups of substances, therefore, have entirely escaped previous discovery, because all the other investigators who have so far attempted to isolate these vitamins have relied exclusively on curative experiments. Therefore, it appears certain that there are several groups of chemical substances that are capable of protecting fowls against polyneuritis gallinarum."

In their attempts to discover a better method for isolating the base, Vedder and Williams also tried the method of Suzuki, Shimamura and Odake but do not seem to have regarded it as constituting any marked improvement over the method of Funk. The method noted above was also abandoned as a quantitative method as the greater part of the curative base was lost during the final precipitation with silver nitrate in the presence of barium hydroxide. A method which proved more successful consisted in neutralizing a quantity of unhydrolyzed extract of rice polishings with barium hydroxide, adding an excess of barium acetate, treating the precipitate thus obtained with five per cent sulfuric acid for three hours and filtering. The filtrate after the removal of the excess of sulfuric acid with barium carbonate proved to have retained practically all of the curative power of the original extract as shown by curative experiments with fowls suffering from advanced polyneuritis.

In the same year Cooper (1913a) described the preparation from animal tissue of a substance which cures polyneuritis in birds:

> Horseflesh was minced, dried at 30° C. before a fan and ground, and the dry powder (weight 4000 grams) extracted at 37° with absolute alcohol on a shaking machine. The filtered extract was evaporated at 40° in vacuo. The extract (weight 500 grams) was found to possess curative properties for pigeons affected with polyneuritis, 4 grams being sufficient to bring about complete recovery within 24 hours. The extract was then treated with an excess of ether and the mixture allowed to stand in the cold for 12 hours. The ether dissolved the fats and lipoids, but left un-

dissolved a considerable amount of a white substance. This was washed thoroughly with ether and then tested on neuritic pigeons. Doses of 0.3 gram were sufficient to bring about complete recovery within 12 hours. The ether-soluble fraction was only very slightly active.

The ether-insoluble fraction was next treated with alcohol, by which a large portion was dissolved. The alcohol-insoluble residue contained both organic and inorganic material, but possessed no curative properties. The alcohol-soluble fraction, which was curative, was treated with excess of ether. This caused the separation of a yellow syrup (yield 50 grams) which was completely soluble in water, and 0.2 gram of which was sufficient to cure polyneuritic pigeons. The aqueous solution of the syrup was allowed to stand in a vacuum desiccator and a white crystalline substance gradually separated. This proved to be carnine and possessed no curative properties. The filtrate, which was highly curative, was next treated with finely powdered lead acetate until there was no more separation of a flocculent precipitate and the mixture was allowed to stand for 12 hours. The precipitate was filtered, washed with water at 35°, decomposed with sulfuric acid, and the excess of acid removed by the careful addition of barium hydroxide. The filtered solution possessed no curative properties. The filtrate from the lead acetate precipitation was freed from lead by careful treatment with dilute sulfuric acid and was left very slightly acid. It possessed curative properties. It was next treated with silver nitrate, which produced a copious, yellowish-white precipitate. This was filtered off, decomposed by hydrochloric acid, the resulting solution neutralized and found curative. The filtrate from the silver nitrate precipitation was also curative, but its content of active substance was completely precipitated by silver nitrate when barium hydroxide was added. By carrying out several animal experiments it was found that at least three-fifths of the total amount of antineuritic substance present in the filtrate from the lead acetate precipitation was precipitated by the addition of silver nitrate only, and about one-fourth was carried out of solution by the subsequent addition of barium hydroxide. The remaining three-twentieths was probably destroyed by alkali.

The residue obtained by evaporation of the curative solution resulting from the decomposition of the first silver nitrate precipitate with hydrochloric acid was next extracted with chloroform. Only a small amount of inactive substance was extracted. The active substance was thus insoluble in chloroform; also insoluble in benzene and in ethyl acetate. It was destroyed by standing in ammoniacal solution; was not destroyed by hydrogen sulfide but appeared to be largely lost by adsorption on precipitates of metallic sulfides.

Cooper (1914b) made use of acetone precipitation in the preparation of antineuritic substance from cardiac muscle; and in this year also made the first use of autolyzed yeast as a starting point for the isolation of the antineuritic vitamin (1914).

In a paper published in June 1913, Funk (1913b) gave the results of further studies upon the chemistry of the vitamin fraction of yeast and rice polishings. By recrystallization from alcohol of the active fraction of yeast prepared by his first method he obtained three substances one of which had the composition $C_{42}H_{19}O_9N_5$, one $C_{29}H_{23}O_9N_5$, while the third appeared to be nicotinic acid. The vitamin fraction from rice

polishings yielded nicotinic acid and a substance to which the formula $C_{26}H_{20}O_9N_4$ was assigned. The work of Suzuki et al was discussed in this paper and in greater detail in one by Drummond and Funk (1914) in which were reported the results of an extended study of the phosphotungstate precipitate from rice polishings obtained according to Funk's earlier method. They were not only unable to obtain the active products mentioned by the Japanese investigators but failed also in isolating the curative substance of rice polishings by an extension of their previous method which had pointed to success. From an exhaustive fractionation of the phosphotungstic acid precipitate they succeeded in isolating comparatively large amounts of choline, nicotinic acid and betaine, together with small amounts of the purines, guanine and adenine, and traces of a substance which they considered might possibly be guanidine.

The substance to which Funk had previously assigned the formula $C_{26}H_{20}O_9N_4$ and which with nicotinic acid had been obtained from the portion containing practically the whole of the curative substance was definitely proved to be also nicotinic acid. "All trace of the curative substance had disappeared and a fraction which originally had shown very marked curative properties now consisted of nothing other than nicotinic acid which possesses very slight action. All attempts which we have made to isolate the elusive curative substance from this fraction, in which it originally occurs have failed." This admission throws doubt upon Funk's earlier isolation of a vitamin of melting point 233° and gives point to Barger's suggestion that this may have been only nicotinic acid contaminated with the active substance.

That Funk and Drummond were at this time evidently of the opinion that there is probably a decomposition of the active substance into nicotinic acid is shown by their statement: "With regard to nicotinic acid, we ourselves lean toward the belief that its occurrence in rice polishings has some relationship to the occurrence of the curative substance and that it may possibly even be a degradation product of the active body, but in the case of choline we firmly believe that it exists as such and in comparatively large amounts in the polishings."

Seidell (1916), adopting a method used by Lloyd in working with alkaloids, found that Lloyd's reagent, a special form of

fuller's earth (hydrous aluminum silicate) effectively adsorbed the vitamin from autolyzed yeast solution. When 50 grams of the fuller's earth per liter of the autolyzed yeast solution were used the vitamin was practically all adsorbed and the solid could then be washed with successive portions of very dilute acid, water and small amounts of alcohol, and finally dried in a vacuum desiccator, thus affording a stable form of the vitamin. Both preventive and curative experiments were made with this material. Prompt and effective cures of completely paralyzed pigeons resulted from 0.05 gram doses of the activated solid corresponding to 1 cc of the original yeast filtrate. The same amount fed on alternate days to pigeons on a polished rice diet proved sufficient to keep the birds in normal health and weight.

Williams (1916 a) published the results of unsuccessful attempts at the isolation of the vitamin from rice polishings by various modifications of Funk's original method. He obtained a very slight yield of crystals melting at 233° which were curative in doses of 10 to 20 milligrams. An amorphous material also gradually separated which was curative in doses of 20 to 30 milligrams and proved effective in treating cases of human beriberi. After exhausting every resource to avoid mixed deposits from the final liquor and to increase the yield of needles melting at 233°, he abandoned the hope of isolating large quantities of an individual vitamin from rice polishings and decided to attempt the synthesis of curative substances which might throw light on the nature of the vitamin. The facts adduced by Funk and others seemed to Williams to point to the pyridine ring as the most promising nucleus. The most important facts in favor of this choice are the occurrence of nicotinic acid with the vitamin in several natural substances and the stability of the vitamin on heating with sulfuric acid.

Nicotinic acid, trigonelline, and p-oxynicotinic acid given by the mouth to neuritic fowls caused little improvement, but some prolongation of life. The hydrochloride of the methyl ester of nicotinic acid brought about marked, but temporary improvement.

Later in the same year Williams (1916 b) published the results of experiments upon the antineuritic properties of some synthetic hydroxypyridines. These were selected on account of the fact that antineuritic foodstuffs when treated with phospho-

tungstic acid and alkali gave the blue color produced by substances containing hydroxy groups in the benzene ring. Polyneuritic pigeons were given doses of 10 to 100 milligrams by intramuscular injections. In some cases similar doses were administered by mouth as a supplementary test. The substances tested included nicotinic, cinchomeric, quinolinic, 6-hydroxynicotinic and citrazinic acids, α-hydroxypyridine, glutazine, 2,4,6-trihydroxypyridine and its anhydride, and finally 2,3,4-trihydroxypyridine and the so-called tetrahydroxypyridine.

Only α-hydroxypyridine, 2,4,6-trihydroxypyridine, and 2,3,4-trihydroxypyridine were found to have curative power. Moreover, these compounds were curative only when freshly prepared and invariably lost their curative properties on standing. As there was no evidence of decomposition it seemed probable that the change was due to isomerization. A study of the isomeric forms and curative properties of α-hydroxypyridine showed that of the two crystalline forms one exhibited activity and the other did not. Similar results were obtained with β- and γ-hydroxypyridine, leading Williams to state: "The antineuritic properties of these substances suggest that an isomerism is at least partially responsible for the instability of the vitamin in foodstuffs and that the antineuritic property may be inherent in the potentiality of this type of isomerism. We may not conclude that vitamins are necessarily hydroxypyridines since a similar isomerism may exist in substances containing other heterocyclic nitrogenous nuclei which are known to occur widely as constituents of animal tissue."

With this conception in mind attention was directed to the natural antineuritic substances of yeast. Starting with activated fuller's earth Williams and Seidell (1916) attempted to separate the vitamin from it by extraction with acidified aqueous alcohol but failed in this attempt on account of simultaneous extraction of aluminum compounds. On shaking the activated fuller's earth with a five per cent solution of sodium hydroxide in dilute alcohol and evaporating the extract, they obtained a crystalline antineuritic substance, the physiological action of which was apparently not due to adhering mother liquor. In attempts to purify this substance further by recrystallization its antineuritic properties were lost; and the resulting product was found to be identical with adenine. On heating 10 milligrams of this sub-

stance with one cubic centimeter of absolute alcohol in a sealed tube at 180° for three hours it acquired antineuritic properties and the power to give a blue color with the Folin-Macallum phosphotungstic sodium carbonate reagent. These results are thought to be in harmony with the conclusions of the previous paper and to suggest the probability that "an isomer of adenine is the chemical entity responsible for the characteristic physiological properties of the vitamin under investigation."

Voegtlin and White on the other hand reported (1916) that they were unable to cause adenine to acquire antineuritic properties and that there probably exists no relation between adenine and the antineuritic vitamin.

In a third paper Williams (1917) described further work with synthetic substances from which he concluded that "the curative form of α-hydroxypyridine is a pseudo base and that a structure conforming more or less closely to the type of a betaine ring is probably an essential characteristic of antineuritic vitamins." Attention was called to the theoretical possibility of the existence of such a substance in some of the simpler nitrogenous constituents of animal tissues especially in the nuclein bases. It was also suggested that nicotinic acid may exist in a betaine form and that the curative properties of the vitamin fractions of yeast and rice polishings may have been due in part to this isomeric form of nicotinic acid.

Harden and Zilva (1917) repeating Williams' experiments with α-hydroxypyridine were able to confirm the chemical properties of that substance as reported by Williams but were unable by its use to effect a cure or even an improvement in the condition of polyneuritic birds. Pure adenine as well as adenine treated with sodium ethylate in a sealed tube for five hours at 100°, also yielded negative results in agreement with Voegtlin and White but in disagreement with Williams and Seidell.

During the past five years vitamin investigations have in general followed other lines and but few further attempts at isolating the antineuritic vitamin have been reported. In May, 1920, Myers and Voegtlin published the results of an investigation covering a period of several years. They reported that olive oil and oleic acid remove the antineuritic substance from autolyzed yeast filtrate. Autolyzed yeast was however finally abandoned as a starting point for the isolation of the anti-

neuritic vitamin as being unsatisfactory on account of its complexity. Likewise mastic, Lloyd's reagent and ferric phosphate were found unsatisfactory adsorbing agents on account of their lack of specificity in the adsorption of basic materials.

The method finally adopted consisted in the extraction of dried brewers' yeast by acid methyl alcohol and precipitation of the purine fractions by hot silver acetate, and of the active substance by barium hydroxide and silver acetate. On decomposition of this precipitate in acid medium by hydrogen sulfide followed by subsequent removal of hydrogen sulfide in vacuo and finally sulfuric acid by lead acetate, the filtrate was found to have lost little of its activity. Mercuric sulfate formed in this filtrate a pale yellow inactive precipitate containing most of the histidine fraction. From the filtrate after removing this precipitate the active substance was precipitated by absolute alcohol. This precipitate was decomposed with hydrogen sulfide and the filtrate freed from sulfuric acid and lead as before. The filtrate, after concentration in vacuo at low temperature, gave a purple solution with ninhydrin, a negative biuret test, no precipitate with picric acid, but gave a heavy precipitate with phosphotungstic acid and was highly curative to polyneuritic pigeons. The solution on further concentration in vacuo over soda-lime yielded spindle-shaped crystals which were active as long as the mother liquor was present. On drying, the crystals changed from spindles to prisms and became inactive. When the prisms were dissolved in a relatively large volume of water and again allowed to crystallize, spindle-shaped crystals were again formed. While realizing that the activity may be in the non-crystallizable mother liquor as well as in the crystals, Myers and Voegtlin are of the opinion that there are at least two substances in the final solution both of a distinctly basic character. One of the impurities is a histamine-like substance as shown by the positive Pauly reaction.

Hofmeister (1920) has reported briefly an attempt to separate the antineuritic substance of rice polishings as follows:

The rice polishings are first shaken with alcohol, the alcohol removed by distillation in vacuo, the syrupy residue acidified with hydrochloric acid to precipitate the fatty acids which are then shaken with ether; the aqueous solution evaporated to a thick sirup and again shaken with alcohol, the clear aqueous solution made slightly alkaline with sodium carbonate and precipitated with bismuth potassium iodide. The precipitate, which at this point contains the choline fraction, is filtered, the filtrate decomposed with one-tenth volume of 20 per cent hydrochloric acid and again precipitated with bismuth potassium iodide. This precipitate, called the oridin fracton, is filtered off with strong suction, triturated with silver carbonate and again filtered. The slightly alkaline silver-containing filtrate is made slightly acid with hydrochloric acid, and, after removal of the precipitated silver chloride, is evaporated to dryness in vacuo at a low temperature yielding an active, slightly colored, deliquescent salt. On treatment with gold chloride a yellow double salt is formed which on analysis corresponds to the formula $C_5H_{11}NO_2.HCl.AuCl_3$.

This salt on decomposition yields a free base isomeric with betaine and valine and probably containing the pyridine or pipiridine groups, but which possesses no antineuritic properties.

Hofmeister evidently favors the view that this base is an isomeric form of the active antineuritic substance.

In April, 1921, Seidell published a preliminary note describing the preparation of a stable silver vitamin compound from activated fuller's earth. This is extracted with saturated aqueous barium hydroxide solution in the proportion of one liter per 100 grams of the activated fuller's earth. After shaking the mixture vigorously for three minutes the solid is removed as rapidly as possible, preferably by means of a De Laval cream separator. The nearly clear liquid is immediately acidified with a slight excess of concentrated sulfuric acid, a moderate excess of powdered barium carbonate is added to remove the excess of sulfuric acid and the mixture filtered after about thirty minutes. To the filtrate nearly saturated lead acetate solution is added to complete precipitation. After filtering and freeing the filtrate from the excess of lead with hydrogen sulfide the resulting filtrate is evaporated rapidly under reduced pressure with removal from time to time of an amorphous white precipitate which begins to separate when the volume becomes small. When this ceases to form the extract can be evaporated to dryness in a vacuum desiccator and in this condition appears to retain its antineuritic properties indefinitely.

On diluting to a volume of 25 cubic centimeters an amount of the crude vitamin extract prepared from 300 grams of activated fuller's earth and gradually adding an almost saturated aqueous silver nitrate solution a voluminous silver precipitate is obtained. On adding to the liquid obtained in centrifuging and washing this precipitate an excess of fairly concentrated aqueous ammoniacal silver nitrate solution another voluminous silver precipitate forms. This second precipitate, as tested by feeding to pigeons on a polished rice diet, is highly antineuritic. It can further be purified of an easily crystallizable non-vitamin compound by adding a slight excess of hydrochloric acid to a suspension in water, removing the silver chloride formed, and slowly evaporating the clear aqueous solution in a vacuum desiccator until crystals cease to form. On removing these crystals, which are inactive, and repeating the precipitation with silver nitrate followed by ammoniacal silver nitrate, a voluminous silver precipitate is again formed but free from the crystallizable inactive fraction. This compound, the yield of which in the dry state

is 0.7 gram from 300 grams of "activated" fuller's earth, when administered in doses of 0.004 gram on alternate days to pigeons on a polished rice diet, prevented the development of polyneuritic symptoms. The birds, however, showed a slight decrease in weight, possibly as a result of the absence of another essential vitamin. "Whether the silver vitamin precipitate obtained as described above is a pure compound is of course not known at present. It is possible that two or more substances are at present in combination with the silver and that different samples which are prepared will vary somewhat in composition. It is believed, however, to be an exceptionally favorable product on which to concentrate efforts toward the identification of the antineuritic vitamin."

At the meeting of the American Chemical Society in September 1921, Seidell (1921) reported further study of the crystalline products obtained in his attempts to isolate the antineuritic vitamin, and Williams (1921) reaffirmed much of his earlier work upon the antineuritic properties of substances of known structure and continued the development of his theory that antineuritic activity may be connected with isomerism of the type above described. Polyneuritic pigeons showed substantial improvement when treated with β-hydroxypyridine or with β-methylpyridine. Protective experiments are regarded by Williams as much more convincing than curative experiments. In protective experiments trimethyluracil and 4-phenylisocytosine gave some evidence of antineuritic property. Williams concludes this (1921) paper as follows:

"Whether this result or any of the physiological results so far obtained with synthetic substances have any real significance must be left to the reader's judgment. However, the solubilities, chemical reactions and natural occurrence of vitamin B so far as known, agree very closely with the pyrimidine bases, a class of substances known to be capable of a very delicate desmotropism. In view of these facts any suggestion of physiological activity in synthetic preparations of this group or its allies ought not to be lightly dismissed. The writer believes that vitamin B eventually will be found to be a cyclic nitrogen compound with an oxygen substitution in the ring and capable of existence in a betaine configuration. If the work on synthetics offers any useful suggestions as to manipulation in the isolation or identifi-

cation of the vitamin from natural sources it will have served an adequate purpose."

The various attempts at isolating the antineuritic vitamin have been given in considerable detail as confirming by the work of several investigators in different laboratories the existence of a previously unknown chemical substance of marked antineuritic potency, and as throwing some light on its probable molecular structure and at least on its chemical behavior. The methods of extraction and precipitation have in general been those commonly used for isolating the simple natural bases. The precipitation with silver nitrate in slightly alkaline solution still further limits the possibilities among known substances of this group. That the material diminishes in activity with each successive fractionation and that in most cases the products which have shown more or less activity by physiological tests have been found on further examination to yield well known compounds such as nicotinic acid, adenine, guanine, etc., indicate either that transformation from active to inactive substances takes place as suggested by Williams or that the vitamin tends to adhere to substances of this nature. Drummond in 1917 after unsuccessful attempts to isolate the vitamin from yeast by various of the well known methods concluded that "the lack of success in such attempts is due not so much to instability of the vitamin as to the losses which occur through the readiness with which the vitamin is adsorbed by precipitates and thus lost in the attempts to purify it." The adsorption of the active material by fuller's earth is an illustration of this property to a marked degree. Possibly other manipulations with resulting colloidal precipitates may involve this phenomenon and thus tend to diminish the quantity and resulting activity of the purified product.

During the same years in which the work on the antineuritic vitamin summarized above was being published, evidence was also accumulating that either this or a similar substance has a most important influence upon growth and plays an essential part in normal nutrition at all ages.

The Water-Soluble Vitamin (Vitamin B).

Osborne and Mendel in 1911 and again in 1912 pointed out the superiority of their experimental rations containing "protein-

free milk" over mixtures of purified proteins, fats, carbohydrates and salts, as food for growing animals, even though the purified food substances were themselves derived from milk.

In 1913 they developed the point more fully giving experimental evidence which pointed plainly to the presence in milk of a water-soluble substance important for growth and adult nutrition and different from any of the known constituents of the diet. Their chief evidence on these points at that time was as follows: Young rats fed solely upon milk food (paste consisting of milk powder, 60 per cent; starch, 12 per cent; lard, 28 per cent) not only grew from infancy to full maturity but gave birth to litters of normal young which in turn have thriven on diet precisely like that furnished to their parents. Therefore this food contains all that is essential for both growth and maintenance.

Mixtures of starch, lard, purified protein from milk, and a salt mixture made in imitation of milk ash, never supported growth. But on mixtures of purified protein, lard, starch and "protein-free milk," young rats grew well for 60 to 100 days or more; when finally there was a sudden decline and death followed unless a change was made in the diet.

The observation that the nutritive decline which eventually sets in on such a diet can be averted and growth renewed by the addition of butter fat to the diet led to the conclusion that a substance exerting a marked influence upon growth is present in butter and tended at the time to concentrate attention upon this factor rather than to emphasize the presence of another growth-promoting substance in the protein-free milk. In December, 1914, Mendel in a lecture on *Nutrition and Growth* delivered before the Harvey Society of New York stated that: "It is not unlikely, to speak conservatively, that there are at least two 'determinants' in the nutrition of growth. One of these is furnished in our 'protein-free milk' which insures proper maintenance even in the absence of growth. When this was fed we have maintained rats without growth for very long periods. Without this 'determinant' (as, for example, in diets of isolated food substances containing artificial substitutes for 'natural' protein-free milk) the special components of butter fat or cod-liver oil or egg fat induce only limited gains at best. Another 'determinant' is furnished by these natural fats."

Meantime Hopkins (1912) had published the results of his discovery dating back to 1906 of the remarkable effect of the addition of small amounts of milk to a diet of purified food-stuffs and had drawn the conclusion that certain natural foods contain an alcohol-soluble, growth-promoting substance or substances. Since both of the substances discussed by Mendel in the paragraph above quoted are soluble in alcohol, the work of Osborne and Mendel was in accord with that of Hopkins and carried the subject further in showing the existence of two factors in the problem.

A paper published by McCollum and Davis (1915) did much to advance the view that "there are necessary for normal nutrition during growth two classes of unknown accessory substances, one soluble in fats and accompanying these in the process of isolation of fats from certain foodstuffs and the other soluble in water, but apparently not in fats." As the result of a study, by means of feeding experiments with young rats, of the dietary deficiencies of rice, it was found that polished rice could not be supplemented so as to produce a ration which will induce growth by the addition of purified protein, fats which possess the growth-promoting property, and salt mixtures; but that the addition of quantities of wheat embryo or of milk powder as small as two per cent of the food mixture of polished rice, casein, salts and butter fat furnished enough of an essential accessory to induce growth. This substance essential for growth was shown to be present in the water and alcohol extracts of wheat embryo and of egg yolk and to be apparently stable to heat. That it is not identical with the growth-promoting constituent of butter fat was shown by the fact that the addition of 20 per cent of butter fat to the basal ration did not induce growth unless the other accessory was supplied nor did the addition of the water-soluble accessory suffice to support growth in the absence of the fat-soluble accessory. That the amounts of the water-soluble factor necessary to induce normal growth are very minute was shown by the fact that amounts of alcohol extract of wheat embryo carrying as little as 0.6 gram of solids and 0.0095 gram of nitrogen, equal to 0.33 per cent of the total nitrogen of the ration, sufficed to induce normal growth.

Attention was also called to the probability that lactose of the ordinary purity may be contaminated with sufficient of the

water-soluble growth-promoting substance to cause pronounced increase in body weight of animals whose ration was otherwise adequate. Later in the same year McCollum and Davis (1915 a) reported that supposedly pure casein as well as lactose may contain appreciable amounts of the water-soluble growth-promoting substance. Casein was therefore purified by twice repeated precipitation, followed by washing, drying and grinding. With casein prepared in this way, together with dextrin, butter fat and salts, they obtained no appreciable growth even during the first month. Nor was growth obtained in the presence of excess of the water-soluble factor with absence of the fat-soluble factor. Thus an explanation was afforded for the observations previously noted by Hopkins and Neville (1913) and others that when purification of the various ingredients of an artificial dietary is carried to a further degree than usual the animals decline at a much more rapid rate than when fed upon a similar diet which is less pure.

Shortly after these papers Drummond (1916) published more detailed evidence leading to the same conclusion that lactose refined to a degree ordinarily regarded as pure is likely to carry considerable amounts of a growth-promoting substance soluble in water and alcohol and not destroyed by exposure to 100° for six hours. This helped to explain the discordant results obtained by various investigators in growth experiments in which lactose had been a part of the experimental dietary and to make more convincing the proof of the indispensability for normal nutrition of the water-soluble and the fat-soluble vitamins.

Similarity in occurrence and properties of the water-soluble and antineuritic vitamin soon led to the suggestion and belief on the part of most workers that these two are identical.

McCollum and Kennedy (1916) stated in unqualified terms that the water-soluble B essential to growth is the same substance which prevents and cures polyneuritis and that it may be extracted from fat-free wheat embryo not only by water and alcohol but also by acetone, benzene and ethyl acetate. They found evidence of the presence of this substance in the juices of potato and cabbage as well as in extracts of wheat and oats.

McCollum, Simmonds and Pitz (1916) concluded from the results of their rat-feeding experiments, as had Andrews (1912) in his study of human beriberi, that vitamin B passes into the

milk only about in proportion as it is supplied by the food of the mother.

Funk and Macallum (1916 a) attempted to concentrate the water-soluble growth-promoting vitamin by such methods as Funk had used in his studies with experimental beriberi. They found that the growth-promoting substance was almost entirely precipitated by means of phosphotungstic acid and while their results did not entirely parallel those of Funk they concluded that the water-soluble growth-promoting substance is analogous to, if not the same as, the antineuritic vitamin.

Eddy (1916) found in sheep's pancreas a water-soluble growth-promoting vitamin which was precipitated by phosphotungstic acid and extracted from the phosphotungstate precipitate by the amyl alcohol method of Jacobs. The fact that this substance was also removed from solution by Lloyd's reagent still further suggests its identity with the antineuritic vitamin.

Osborne and Mendel (1917 a) discussed further the rôle of vitamins in the diet. To promote normal growth in white rats with dried yeast as the sole source of water-soluble vitamin required, in their experiments, the presence of about two per cent of yeast solids in the food mixture. They expressed the opinion that water-soluble vitamin exerts a favorable influence upon metabolism, improving the general condition of the animal and thus increasing the appetite, rather than merely rendering the food mixture more palatable and thus inducing the animal to eat more. Subsequently they demonstrated experimentally that an animal's appetite for vitamin-free food can be improved by feeding the vitamin separately in the form of a small amount of dry yeast or dried spinach. As to whether the water-soluble vitamin with which they were dealing was the same substance which prevents and cures polyneuritis, Osborne and Mendel at this time wrote: "Whether or not the antineuritic component is identical with the growth-promoting one is a question which as yet has received no definite answer, although Funk and Macallum think that their results 'indicate that the growth-promoting substance is analogous to and possibly identical with the beriberi vitamin' but that considerably larger quantities of vitamins are necessary for stimulating growth than for curing beriberi."

Osborne and Mendel conclude this paper with the statement that yeast as a source of water-soluble vitamin had not given as

good results as were obtained from the use of "protein-free milk." "Although some of the animals brought up on the yeast-containing foods have given birth to young, thus far none of the latter has been reared." The work of Osborne and Mendel made it plain that this water-soluble vitamin so often referred to as "growth-promoting" is certainly essential to normal nutrition at all ages.

In another paper published later in the same year Osborne and Mendel (1917 b) in calling attention to the fact that up to this time milk and pancreas were the only products of animal origin that had been tested for the presence of water-soluble vitamin say "indirect evidence that animal tissues may contain this hormone is afforded by studies of the protective, curative or antineuritic properties of some of these in relation to beriberi. That the substance which induces the remarkable recoveries which have been described in these cases is identical with the water-soluble hormone which is so essential for growth and maintenance is as yet merely a matter of conjecture." In the experimental work reported in this paper it was shown that muscle tissues and meat extract contain but little of the water-soluble growth-promoting vitamin but that liver contains a large proportion of this substance. It was pointed out that these results parallel the findings of Cooper in respect to the relative amounts of antineuritic vitamin in muscle and liver. Attention was also called to the fact that both liver and pancreas in contrast to muscle tissue are exceptionally rich in glandular cells.

Drummond (1917), following attempts to isolate and determine the chemical nature of the water-soluble vitamin, concluded that it was either identical with the antineuritic vitamin or that the two substances were closely related members of the same class. Drummond's feeding experiments were conducted upon young black rats. The various components of the basal diet (casein 18, starch 40, agar 5, salt mixture 5, sucrose 17, and butter fat 15 parts) were carefully purified before use by extractions designed to remove all traces of the water-soluble growth-promoting factor. To this basal ration were added varying amounts of the material to be tested for the presence of the vitamin. The addition of 6 per cent dried yeast gave better growth than 3 per cent, but when the percentage was increased to 12 no further improvement was noticed. The growth factor

was not removed from dried yeast by extraction with absolute alcohol or ether nor appreciably from yeast extract by absolute alcohol but proved more soluble in 70 per cent alcohol. Treatment of dried yeast by heating to 100° for 30 minutes did not affect its activity, while injury but not complete destruction resulted from autoclaving at 120° for 30 minutes. The activity of the yeast extract was not appreciably diminished by boiling with dilute (1 per cent) hydrochloric acid for 12 hours nor by treating with 5 per cent sodium hydroxide at room temperature for 24 hours. Treatment with hot 5 per cent sodium hydroxide for 5 hours tended to destroy its activity.

Attempts to isolate the active substance by methods similar to those used in the study of the antineuritic vitamin proved even less successful, great loss of activity occurring during the fractionation. This, as has been stated earlier, was attributed by Drummond not so much to instability of the vitamin as to its ready adsorption by colloidal precipitates.

Drummond in 1918 published his second paper on vitamin B dealing chiefly with its distribution in the animal body and its effect upon nutrition and nitrogen metabolism. He found the food consumption of rats fed upon a diet deficient in water-soluble B to be low. Increased food consumption was brought about by the addition to the diet of flavoring extracts or of extracts containing vitamin B but growth took place only when the extract contained vitamin B and the extent of growth was within certain limits proportional to the amount of vitamin B added, provided that the diet was adequate in other respects. The length of time that an animal is able to maintain itself on a diet deficient in water-soluble B without suffering serious loss of body weight appeared to be directly proportional to the age at which the restriction is imposed. Actively growing tissues (embryos, tumors, etc.) did not contain appreciable amounts of vitamin B, and desiccated pituitary gland (whole gland and anterior lobe), thyroid, thymus, testicles and ovarian tissues were also found to be deficient in this vitamin.

The only apparent deviation from the normal noted in the nitrogen metabolism of rats on diets deficient in vitamin B was the appearance of creatinuria accompanied by a slow wasting of the skeletal muscles. Drummond was unable to determine the cause of the fatal decline which invariably follows a deficiency

in vitamin B. Symptoms of nerve disorder were found in only three of the many cases studied. Some other observers have noted the occurrence of neuritis much more frequently in such cases.

A further study of the solubility and stability of the water-soluble growth-promoting vitamin was made by McCollum and Simmonds (1918) using white beans or wheat embryo as sources of the vitamin. The technique employed in this and other similar studies consisted in feeding young rats a diet of purified food substances (casein, salt mixture, agar-agar and dextrin) together with 5 per cent of butter fat to supply an abundance of vitamin A. The rats were confined to this diet for about five weeks until they had either become stationary in weight or were declining with evidences of paralysis. The material to be tested for vitamin B was then added to the diet, the animals continuing to decline or responding with growth, according as the material was deficient or rich in this vitamin. This method was considered to show within two weeks whether the preparation under investigation contained vitamin B in significant amounts. As tested by these criteria, it was shown that vitamin B is not extracted directly from beans, wheat germ or pig kidney by ether, benzene or acetone, but is readily extracted in great part by alcohol (95 per cent). After being removed by alcohol it was shown to be soluble in benzene but very slightly soluble in acetone. McCollum and Simmonds conclude that: "The probability that there should be two or more physiologically indispensable substances in what we term water-soluble B, both or all of which should show the same solution relations with three solvents, is relatively small and lends support to our view that the substance which protects animals against polyneuritis is the only essential complex in the extracts described." A few experiments on the stability toward acids and alkalies of water-soluble B in the form of an alcohol solution of wheat embryo were also reported. These tended to confirm similar tests of the antineuritic vitamin in its rapid destruction by alkalies and relatively marked stability toward hydrochloric acid. Nitrous acid had slight if any action, this fact constituting a strong indication that the active substance is neither a primary nor a secondary amine.

Osborne and Wakeman (1919) described experiments in which they sought by fractional precipitation with alcohol of the

water extract of yeast, to concentrate the water soluble vitamin in a fraction and thus obtain it free from all but a comparatively small proportion of the other constituents of the yeast. Instead of submitting the yeast to autolysis as in the work of previous investigators on the antineuritic vitamin, they prepared the original water extract by coagulating the yeast by boiling water. Their experimental procedure was in brief as follows: Several liters of fresh bottom yeast were obtained directly from a brewery, and immediately diluted with ice water, centrifuged and the sediment washed twice more in the same way. The moist washed yeast weighing 264 grams was stirred gradually into one liter of boiling distilled water containing 10 cubic centimeters, or one per cent, acetic acid. After boiling for about two minutes the solids were separated by means of a centrifuge. The water extract thus prepared contained 17.1 per cent of the solids of the yeast and 14.4 per cent of its total nitrogen.

This water extract, although containing less than one-fifth of the yeast solids and only one-seventh of the yeast nitrogen, contained nearly all of the water-soluble vitamin. Daily doses of 17 milligrams of the solids of this extract promoted the recovery and rapid growth of young rats declining on a diet free from water-soluble vitamin. The water extract was then prepared in the same way but in larger quantity from 4.5 kilograms of moist yeast and the water extract after concentration to two liters was poured into three liters of 93 per cent alcohol, making the alcoholic content of the mixture about 52 per cent by weight. The flocculent precipitate which separated was designated as Fraction I. The filtrate and washings from Fraction I were concentrated to 300 cubic centimeters and poured into 1,960 cubic centimeters of 93 per cent alcohol, making the alcohol content of the mixture about 79 per cent by weight. The resulting precipitate (Fraction II) was washed once with 79 per cent alcohol and then twice dissolved in about 100 cubic centimeters of water and reprecipitated by pouring into enough alcohol to make the alcohol concentration 90 per cent by weight. After standing under absolute alcohol and drying over sulfuric acid, a light-colored, friable product was obtained equal to 51.8 grams of dry material or to 37 per cent of the solids of the extract or to 6.2 per cent of the solids of the original yeast. This dried Fraction II contained 7.5 per cent of nitrogen or the equivalent

of 4.5 per cent of the total nitrogen of the original yeast. The ash content of the dry Fraction II was 10.65 per cent.

Numerous feeding experiments showed this fraction to be highly efficient in promoting the growth of young rats when fed as the sole source of water-soluble vitamin and in restoring animals which were declining on a vitamin-free diet. Osborne and Wakeman determined the distribution of nitrogen between humus, ammonia, amino, basic and purine forms in this yeast fraction before and after hydrolysis with results which they interpret as indicating the presence of relatively large proportions of nucleic acid, amino-acids, and peptides. Experiments upon this material, Fraction II, showed it to be very soluble in water, the resulting solution being distinctly acid to litmus. They state that: "Relatively considerable quantities of alkali are needed to produce a neutral reaction to litmus and not a little more must be added before an alkaline reaction results. Only a trace of a precipitate separates from the neutralized solution.

"The unneutralized solution gives a large precipitate with lead acetate. Barium chloride causes only a turbidity, but yields abundant precipitate when the solution is previously neutralized with sodium hydroxide. Silver nitrate does likewise.

"Baryta solution gives a voluminous precipitate which contains about 25 per cent of the solids of the fraction and a relatively small part of its nitrogen. About 25 per cent more of the fraction is thrown out of the alkaline filtrate from the barium precipitate by silver nitrate. This precipitate, when thoroughly washed with baryta solution, contains nearly one-half the nitrogen of the fraction. The aqueous solution of Fraction II when acidified with sulfuric acid yields a very large precipitate with phosphotungstic acid. Mercuric chloride gives a precipitate; copper sulfate gives none. An aqueous or alcoholic solution of picric acid gives a precipitate if enough is added. Whether any one of the above precipitates contains some or all of the water-soluble vitamin remains to be determined.

"Such preliminary observations indicate that a variety of substances are present in this fraction, but give no clue to the nature of the water-soluble vitamin. We have provided ourselves with several kilos of this active yeast fraction and are now engaged in a systematic study of its constituents."

In a further study of this yeast fraction Osborne and Leavenworth (1921) have found that its efficiency as a source of water-soluble vitamin, as indicated by growth experiments with rats, is only slightly if appreciably affected when the material is dissolved in tenth-normal sodium hydroxide and allowed to stand at a temperature of 20° C. from one-half hour to 18 hours, whereas when such a solution stood for 90 hours at 20° its efficiency was very greatly reduced, indicating the destruction of a large proportion of the water-soluble vitamin present. A still larger proportion if not the whole of the vitamin present was destroyed when a solution of the same alkalinity after standing for 18 hours at 20° was heated for one hour at 90°. The authors conclude that in attempting to concentrate or isolate the water-soluble vitamin B, dilute alkaline solutions can be used without materially affecting its activity provided that a low temperature and a short time of exposure to the alkali be employed.

Are the Antineuritic Vitamin and "Water-Soluble B" the Same Substance?

While the studies that have been briefly reviewed above have pointed to the close resemblance or identity of the growth-promoting vitamin B and the antineuritic vitamin, a critical comparison of the literature bearing directly or indirectly on the subject reveals certain dissimilarities which leave the question still unanswered. Such a comparison has been made by Mitchell (1919). After referring to the belief that the antineuritic and water-soluble growth-promoting vitamins are identical he points out that "This belief ranges from positive conviction, through tacit acceptance, to a frank weighing of the probabilities." As his paper constitutes an excellent survey of the literature on the subject with a critical evaluation of the data presented in the various contributions, it will be reviewed at considerable length. According to Mitchell:

"The conclusion that the two vitamins are identical seems to be based upon the following grounds. (1) The distribution of the two substances in natural food products is very similar and the correlation between the actual amounts (in so far as these have been measured by biological tests) found in different products appears to be close. (2) The lack of known sources of water-soluble B in the diet of various species of experimental

animals seems generally, if not always, to result in symptoms of nerve degeneration and paralysis. (3) Extracts of natural food products possessing growth-promoting properties are said to contain very probably only one indispensable vitamin, though supplementing satisfactorily a ration containing no other possible source of antineuritic vitamin. (4) The solubilities of the two vitamins in the common solvents are said to be identical. (5) Attempts at isolating the two vitamins have shown that they possess identical precipitants and adsorbents. (6) The stabilities of the two substances, especially to acids, alkalies, and elevated temperatures, seem to be similar if not identical."

In discussing the first point, attention is called to instances in the literature in which the correlation between the distribution of the antineuritic vitamin and water-soluble B is not close. This seems to be particularly the case in green vegetables, roots and tubers which appear to contain rich or moderate amounts of the growth-promoting water-soluble vitamin (Osborne and Mendel, 1919, 1919a; McCollum, Simmonds and Parsons, 1918a; Sugiura and Benedict, 1918) and small amounts or none at all of the antineuritic vitamin (McCollum and Kennedy, 1916; Chick and Hume, 1916-17; Vedder and Clark, 1912; Chamberlain, Vedder and Williams, 1912). While Mitchell points out that if this relation is abundantly confirmed by future research "it may of itself effectively dispose of any contention of the identity of the two vitamins," he shows that the evidence as it stands does not amount to finality on account of the variability of the vitamin content of vegetables, roots and tubers in different stages of freshness and maturity of the material, the lack of experiments on the antineuritic and growth-promoting properties of the same vegetable samples (see, however, the work of Emmett and Luros beyond), and the unreliability of experimental results in investigations of the comparative antineuritic properties of foods when polished rice, in itself defective in several factors other than the antineuritic vitamin, is used as the basal diet.

Experiments with unpolished rice are considered to afford some evidence of the lack of identity of water-soluble B and the antineuritic vitamin as shown by the observation of McCollum and Davis (1915b) that unpolished rice is adequately supplemented by casein, salt and butter fat, thus implying a sufficiency

of water-soluble B—and of Gibson and Concepcion (1914) that unpolished rice does not furnish complete protection against polyneuritis in pigeons, which, if confirmed, would show an insufficiency of the antineuritic vitamin. The experience of other investigators, however, does not bear out that of Gibson and Concepcion on this particular point.

The second point, that lack of vitamin B in the diet of various species of experimental animals results in symptoms of nerve degeneration and paralysis, is dismissed by Mitchell with references to the observation of Osborne, Wakeman and Ferry (1919) that such symptoms do not always develop on a diet lacking in water-soluble B, and of Hart, Miller and McCollum (1916) that "malnutrition histologically characterized by nerve degeneration may result from the presence of toxic materials in apparently normal food products and in the presence of all known factors essential for continued growth and well-being."

As an illustration of the third point, that similarity in extraction points to the identity of the two vitamins, the statement of McCollum and Simmonds (1918) noted above is quoted. Their argument of the improbability of two or more physiologically indispensable substances possessing the same solubility relations toward several solvents is considered to be of a circumstantial character. "In fact it is quite conceivable that the treatment of natural foods with boiling alcohol breaks up combinations of vitamins with other substances so that the removal of the water-soluble B by this solvent may have been a combination of chemical and purely solvent action."

In reviewing the literature on the solubilities of the water-soluble B and the antineuritic vitamin, both are shown to be soluble in water and dilute alcohol and, generally speaking, insoluble in fat solvents. Water-soluble B is said by Osborne and Mendel (1917 a) and by Drummond (1917) to be insoluble in absolute alcohol, and by McCollum and Simmonds (1918), only incompletely soluble in 95 per cent alcohol. On the other hand, the antineuritic vitamin has been said to be soluble in absolute alcohol by Eijkman (1911), Funk (1911), Fraser and Stanton (1910) and others who have worked on its extraction and concentration. The conclusion that the growth-promoting factor is of somewhat lower solubility than the antineuritic is thought to be even more clearly shown in the case of acetone and benzene.

In McCollum's laboratory, it has been reported that acetone and benzene extract the antineuritic vitamin from fat-free wheat embryo (McCollum and Kennedy, 1916), but that the same solvents do not extract the water-soluble B to any appreciable extent (McCollum and Davis, 1915 b; McCollum and Simmonds, 1918). After extraction with alcohol, however, water-soluble B appears to be only slightly soluble in acetone but more readily soluble in benzene.

"From the work quoted, therefore, the evidence lends support to the conclusion that the water-soluble B is not extracted from food materials to any appreciable extent by either acetone or benzene, and that when extracted it is still only slightly soluble in acetone, though readily soluble in benzene. The antineuritic vitamin, however, seems to be readily extracted from wheat embryo by acetone and benzene, and to be readily soluble in both of these solvents."

In discussing the attempts at isolating the two vitamins Mitchell criticises the work of Funk and Macallum (1916 a) and of Eddy (1916) on the ground that the basal rations used in making the biological tests, besides their deficiency of water-soluble B "could not have contained more than a trace of fat-soluble A." The results of Drummond (1917) while supporting "the view that water-soluble B and the antineuritic vitamin are identical do not constitute a demonstration. The lack of food intake records complicates their interpretation while the large losses of vitamin as soon as precipitation was resorted to find no parallel in analogous work with the antineuritic substance." "The ready adsorption of water-soluble B by precipitates of all descriptions postulated by Drummond, in explaining the large losses during chemical manipulation, does not seem to be a property of the antineuritic vitamin. Thus, Emmett and McKim (1917) show that while this vitamin is adsorbed by fuller's earth and Lloyd's reagent it is not adsorbed by the kieselguhrs or infusorial earths, indicating a selective adsorption by the former. Being readily dialyzable, there seems to be no compelling reason for believing it to be indiscriminately adsorbed by precipitates of all kinds."

With regard to the stability of the two vitamins, evidence is cited that both are stable to acids and to even concentrated alkalies at room temperature. At the boiling temperature the

antineuritic vitamin appears to be rapidly destroyed by alkali (Steenbock, 1917), while the growth-promoting vitamin is more stable although the results with alkalies are somewhat confusing (Drummond, 1917; Osborne, Wakeman and Ferry, 1919; McCollum and Simmonds, 1918; and Daniels and McClurg, 1919).

As to stability toward heat, the evidence is also conflicting for temperatures above 120°, although a greater stability of the growth-promoting vitamin is again indicated (Chick and Hume, 1917 b; McCollum and Davis, 1915 c; McCollum, Simmonds and Pitz, 1917; Daniels and McClurg, 1915; and Drummond, 1917).

Mitchell concludes that there seems to be very good reason for doubting the identity of the two vitamins but that in settling the question definitely there is need of quantitative experiments in which the same materials are tested for their growth-promoting and antineuritic properties.

Emmett and Luros (1920) have recently reported an investigation following this plan, the results of which cast further doubt upon the usual assumption that the antineuritic and water-soluble growth-promoting vitamins are identical. The particular phase of the problem considered was the stability of the water-soluble vitamin to heat. A compilation of the available data on this point, with some consideration of the possibility that the amount of ration consumed might carry an excess of the vitamin beyond minimum requirement and so mask partial destruction, showed that the antineuritic and the growth-promoting vitamins as measured by polyneuritic pigeons and young rats respectively, were fairly stable at temperatures around 100-105° C., while at higher temperatures the antineuritic vitamin seemed to be less stable to heat and alkali than the rat growth-promoting vitamin. Comparable results were obtained in a series of experiments in which the same source of water-soluble vitamin, namely unmilled rice, was used for studies on polyneuritis in pigeons and the rate of growth in young rats.

In the case of pigeons, the unmilled unground rice constituted the sole food while with rats the unmilled ground rice was supplemented with lactalbumin, salt mixture, butter fat and lard to form a balanced ration for growth. The results obtained in feeding experiments in which the rice had been subjected to different heat treatments showed that "while the antineuritic

vitamin is stable to heat at 120° C. and 15 pounds pressure for 1 hour, partially altered by heating in the air oven at 120° for 2 hours and totally destroyed at 120° and 15 pounds pressure in 2 and 3 hours, the water-soluble B vitamin (rats) appears to be stable to heat at these same temperatures, that is, it is not distinctly or totally broken down. Whether this vitamin was slightly destroyed could not be definitely ascertained due to the lack of quantitative methods.

"These findings suggest, tentatively at least, that the antineuritic (pigeons) and the water-soluble B (rats) vitamins are not the same, and that it would be better to consider them as being different until there is further proof to the contrary."

Recently Funk and Dubin (1921) have embraced the view that what has heretofore been called vitamin B consists of a mixture of two vitamins—first, the antineuritic substance readily removed from autolyzed yeast by adsorption upon fuller's earth, to which they propose that the designation vitamin B shall hereafter be confined; and second, the water soluble substance less readily adsorbed by fuller's earth and exerting a specific growth-promoting influence upon microörganisms and which they propose to call vitamin D. Their experimental evidence is as yet admittedly not sufficient to permit of more definite discussion.

Before this question can be answered satisfactorily it will probably be necessary to have a considerable number of parallel experiments in which the same specimens of foods or extracts are fed for the prevention of polyneuritis and for the support of normal growth, and in both cases with quantitative records of the intake as well as due regard to the age and size of the experimental animal. The rations must also be so planned that any material diminution of the B vitamin will actually retard growth. This will probably necessitate the averaging of large numbers of experiments since Osborne and Mendel find large individual variations in the requirement for the B vitamin.

The antineuritic properties should be tested by preventive rather than curative experiments. The results of Williams, of Dutcher, and others have shown that "curative" antineuritic effects can be demonstrated upon polyneuritic pigeons by the administration of a wide variety of substances, not all of which surely would in any case be regarded as identical with growth-promoting vitamin. Furthermore, much uncertainty necessarily

attaches to the results of "curative" experiments through the fact that some distinctly polyneuritic pigeons have been observed to show marked improvement without any treatment.

Since most of the tests of antineuritic properties heretofore made have been by means of curative experiments which we now believe should be confirmed by preventive experiments before final interpretation, and since both extraction experiments and feeding trials need to be made in a more strictly quantitative way than has been customary in the earlier work, it would seem wise to conclude that the evidence thus far available does not prove either that the water-soluble growth-promoting and antineuritic vitamins are the same or that they are different.

It might perhaps be said that the preponderance of evidence thus far available favors the view that the water-soluble growth-promoting vitamin is probably among the substances which may exert antineuritic action.

Physiological Properties.

The changes in the central nervous system induced by the absence of the antineuritic vitamin are so striking and respond so readily to treatment that they have tended to obscure the other manifestations resulting from a partial or total deficiency of this vitamin in the diet. Attention was called to this by McCarrison (1919) in a series of studies conducted at the Pasteur Institute of Southern India on the general subject of pathogenesis of deficiency disease. Working first with pigeons he was able to demonstrate a certain parallelism between the effects of simple starvation and lack of vitamins. In cases uncomplicated by bacterial invasion, both simple starvation and a diet of polished and autoclaved rice brought about loss in weight, a progressive fall in body temperature, and slowing of respiration. The weights of the various organs of the body immediately after death showed atrophy of the thymus, testicle, spleen, ovary, pancreas, heart, liver, kidneys, stomach and thyroid in decreasing order and marked hypertrophy of the adrenals. The pituitary gland and the brain showed no change in the starved pigeons, while on the vitamin-deficient diet a slight tendency to enlargement of the pituitary was noted in the male birds and slight atrophy of the brain in both male and female. The central nervous system as a whole underwent little atrophy, the para-

lytic symptoms being mainly due to impaired functional activity of the nerve cells rather than to their degeneration.

McCarrison places great significance on the hypertrophy of the adrenals for want of vitaminic substances since this occurs both as a result of starvation and of a polished rice dietary. "Endocrinology provides another striking illustration of such hypertrophy, namely, that of the thyroid for want of iodine. The thyroid hypertrophies either because of the lack of iodine in the food or because of its imperfect assimilation due to microbic action in the gastro-intestinal tract, and most commonly when both factors operate together. My researches with regard to beriberi are leading me to a like conclusion concerning the adrenals: these enlarge for want of antineuritic vitamins in the food and probably also in consequence of the inadequate assimilation of these substances—owing to microbic or parasitic action in the gastro-intestinal tract, or for both reasons."

McCarrison's study of the relation between infection and deficiency disease was conducted on pigeons naturally and artificially infected with *Bacillus suipestifer*. The infected birds when fed on polished rice developed symptoms of polyneuritis more rapidly than non-infected birds. Asthenic and fulminatory forms of polyneuritis were much more frequent in the infected birds, which rarely survived long enough to develop cerebellar symptoms. Such symptoms however developed in birds in which infection had been prevented by isolation and immunization. Control birds fed on a liberal diet of mixed grains were in general immune, although exposed to infection. These results are thought to illustrate the influence which infectious agencies probably exert in man under like conditions of dietetic deficiency, beriberi being more frequent among peoples subjected to the attack of innumerable bacterial and other parasitic agencies, to which they are rendered highly susceptible in consequence of a dietetic deficiency.

The general conclusion of McCarrison at this time was that the pathological condition resulting from a vitamin-free diet is due (a) to chronic undernutrition, (b) to derangement of function of the organs of digestion and assimilation, (c) to disordered endocrine function, especially of the adrenal glands, and (d) to malnutrition of the nervous system. "The whole morbid process is believed to be the result of nuclear starvation of all tissue cells,

Even the adrenals, which alone of all organs of the body undergo hypertrophy, show on section changes in some of the cells indicative of nuclear starvation." For this reason the term "nucleopast" (that which feeds the nucleus) has been suggested by McCarrison as a more accurate and apt term than antineuritic vitamin.

While the work which has just been reviewed was concerned with essentially complete vitaminic deficiency, the correctness of attributing the pathological conditions in so far as vitamins were concerned to lack of vitamin B is indicated by McCarrison's second paper of the series in which he reports practically the same results from feeding pigeons autoclaved rice plus onions and butter to furnish vitamins C and A respectively. The only marked difference in the findings was the comparative freedom from edema in the experimental pigeons, thus pointing to the possibility of an anti-edema factor present in the onion or butter (probably in the latter), and also present in unpolished rice. This would seem to suggest the possibility, which has apparently not yet been sufficiently considered, of a connection between lack of vitamin A and edema such as is present in wet beriberi and in the so-called hunger edema.

In the more detailed study of the histological changes in the intestines of pigeons on vitamin-deficient diets, autoclaved rice alone was used as the diet deficient in all three vitamins, rice and butter as the one deficient in B and C, and rice and onion as the one deficient in A and B. The chief microscopical changes observed at autopsy in pigeons on these diets were atrophy and congestion, the latter being more largely confined to the upper part of the alimentary tract, and tending to open the way to systemic infection from the diseased intestine. Interpreted in terms of bowel function the derangements to which these pathological changes may ultimately lead are classified as impairment of the neuromuscular control of the bowel; impaired transport of the intestinal contents along the alimentary canal; impairment of assimilative power; impairment of secretory function; impaired protective resources leading to infection of the mucous membrane of the bowel by pathogenic saprophites or ingested bacteria and to systemic infection therefrom. That these gastro-intestinal lesions are much more deep seated than those of the nervous system is indicated from the observation

that the nervous symptoms present in avian polyneuritis may be rapidly ameliorated and recovered from, and yet the birds die in consequence of gastro-intestinal lesions. In the nervous system the symptoms are considered to be mainly the result of functional disorder, while in the intestine a greater destruction of tissue cells takes place.

From the order of severity in which histological changes occur in different organs, McCarrison concludes that "the organs which suffer most are those which are least essential to the life of the individual. Next in order are the organs of digestion and assimilation, then the organs of excretion, and lastly the organs of internal secretion. That the central nervous system suffers least from the point of view of organic lesions is shown by the rapidity with which the nervous symptoms due to the deficient diet can be controlled or abated by the administration of vitaminic substances. It seems probable that the cellular elements of the organs least essential to the life of the individual are utilized to provide accessory food factors and other nutritive materials for the cells of higher function."

Findlay (1921), in a very carefully controlled comparative study of starvation and beriberi in pigeons and fowls, has confirmed the work of McCarrison to the extent that the histological changes in beriberi were found to be similar in gross manifestations to those of starvation, including hypertrophy of the adrenals, little or no change in the pituitary gland, and atrophy of the other organs. Microscopically they were found to differ from those of starvation in evidence of nuclear degeneration, chromatolysis in the cells of the nervous system, and retention of lipoids in the adrenal cortex. The administration of vitamin B in the form of yeast to birds suffering from beriberi was followed by the removal of lipoids from the adrenal cortex, an increase in the nucleic acid content of the brain, and the disappearance of the paralytic symptoms. On the basis of these findings together with the observations that the nucleic acid content of the organs of the ox is related quantitatively to the vitamin B content of the same organs as determined by Cooper, and that the liver and brains of birds with beriberi show a decrease in nucleic acid content which is more marked in the liver than the brain, the theory of the relationship of vitamin B to nuclear metabolism suggested by McCarrison is advanced still further as follows:

"Vitamin B is essential for the formation of nucleic acid. When the tissue content of vitamin B falls below a certain level the phosphorized lipoid, instead of passing on to the central nervous system is immobilized in the adrenal cortex. The central nervous system begins to run short of nuclear material and a functional paralysis occurs. If the nuclear shortage becomes very acute, the essential nerve centers in the medulla are at length affected and death ensues, but if vitamin B is given in time the lipoid of the adrenal cortex is liberated, the nuclear starvation of the central nervous system disappears and the paralytic bird is restored to health with dramatic rapidity. If the hypothesis which we have suggested be correct, several facts can be viewed in a new light. It becomes apparent why muscular activity, by using up nuclear material in the central nervous system, as evidenced by chromatolysis, causes a shortening of the incubation period of beriberi, why, in fact, any conditions such as growth, entailing an increased demand for nuclear material, should rapidly use up the available supply of vitamin B factor, while starvation in which nuclear activity is reduced to a minimum is not accompanied by symptoms of beriberi." (Findlay.)

These investigations have been reviewed at some length as indicating the profound effect which a qualitatively insufficient diet lacking in vitamin B may have upon the bodily functions, and as offering a possible clue to the nature of the function of vitamin B in metabolism. It should be emphasized, however, that in experiments in which polished rice serves as the sole nutrient aside from the vitamin-containing substances other nutritive deficiencies are involved which may complicate the issue in so far as the physiological properties of vitamin B alone are concerned. Investigations which take this into consideration have been reported recently by Simonnet (1920, 1921).

On the basal diet described on page 75 supplemented by a small amount (0.5 to 1 gram) of dried brewery yeast, pigeons can be kept in approximate weight equilibrium by the necessary quantitative adjustment of the diet. On this same diet minus the yeast, symptoms of polyneuritis developed in from 19 to 49 days, but with no loss in weight during the incubation period such as is the rule on a polished rice diet. On the contrary the general condition of the experimental pigeons during the period before the onset of polyneuritic symptoms is said to be excellent, the appetite good, and the temperature curve normal.

In addition to the classical symptoms of polyneuritis which developed after this preliminary period, Simmonet mentions vomiting (see also Karr), inability to swallow, diarrhea with presence of bile salts in the excretions, and an increase in the output of uric acid. Changes in body temperature are said to follow a rhythmical course apparently connected with corre-

sponding irregularities in respiration. Death follows one of these respiratory crises, "air hunger," unless the vitamin-containing substance is administered. The violence of the symptoms and the rapidity with which they can be ameliorated by the proper treatment are thought to point to "a striking intoxication of certain nerve centers."

Contrary to the results reported by McCarrison, autopsy revealed no microscopic lesions indicating a marked interference with the mechanism of digestion. This is attributed to the difference in the experimental rations. "The fact that with our régime the animals ate until the last day, that it is possible to cure in a permanent manner very severe nerve lesions, and to obtain in series symptoms followed by cure inclines us to think that the intestinal lesions noted by the English investigator (McCarrison) refer to another cause and that the nerve manifestations are purely functional."

These results are thought to show the possibility of eliminating the factor of starvation to a very great extent in deficiency disease and of thus simplifying the study of the question of the identity or non-identity of the growth-promoting water-soluble vitamin and the antineuritic vitamin. Attention is called, however, to differences in the pathological conditions induced in different species of experimental animals by lack of vitamin B. In the pigeon under suitable conditions the nerve lesions appear before evidences of starvation, in the rat the nerve lesions are less marked than the effects of starvation, and in the dog as shown by Karr (1920) the nerve lesions manifest themselves only in those animals which continue to eat until nearly the time of appearance of the nerve symptoms, while others which refuse to eat die of starvation without manifesting nerve lesions.

That starvation may be an important factor in the genesis of pathological conditions induced by lack of vitamin B has been recognized in much of the recent vitamin work. Karr (1920, a, b), working in Mendel's laboratory, has demonstrated failure of appetite, decreased food consumption and eventually polyneuritic symptoms in dogs on diets lacking in vitamin B and prompt recovery on the administration, apart from the diet, of extracts containing the vitamin. Lumière (1920) goes so far as to venture the hypothesis that failure of appetite and consequent starvation is the real cause of the pathological conditions

produced in pigeons on diets of polished rice. In support of this he claims to have produced the classical symptoms of polyneuritis in from 12 to 14 days in pigeons on a diet rich in vitamins but quantitatively insufficient, the daily ration consisting of 4 grams of polished rice, 2 of glucose, and 1 of dried brewery yeast, and to have cured the condition on the subsequent administration of a sufficient amount of polished rice. In an attempt to explain the failure of appetite of pigeons fed on polished rice a comparison was made of the changes taking place in the crops of pigeons forcibly fed on unpolished and polished rice respectively. On opening the crop several hours after the feeding of a large amount of the rice the unpolished grains were found to be thoroughly moistened, and the polished very dry. The author concluded that "pigeons submitted to a régime of polished rice die, therefore, of inanition because they lose their appetite. This loss of appetite seems due principally to the insufficiency of the functioning of the glands of external secretion whose normal activity appears to be due to the presence in foods of substances which excite it. The vitamins intervene to fill the excito-secretory rôle as well as to maintain the tonus of the organs of digestion."

Further work along these lines has just appeared from Mendel's laboratory (Cowgill, 1921 b; Cowgill and Mendel, 1921). Dried brewery yeast, neutralized tomato juice, and alcoholic extracts of wheat embryo, of rice polishings, and of navy beans were employed as sources of vitamin B. All these materials show pronounced curative effects when fed to polyneuritic pigeons and all were found capable of restoring the desire for food in a dog which had lost appetite through being confined to a diet lacking vitamin B. Dogs on diets adequate in other respects but lacking vitamin B usually show loss of appetite in from five to fifteen days and thereafter eat very irregularly if at all. If the dog continued to eat some food it eventually showed symptoms of polyneuritis differing somewhat in appearance from those exhibited by pigeons and fowls. Paralysis of the hind legs resulting first in a peculiar dragging of the feet and then in complete loss of control of the hind limbs was the most characteristic feature in the dog. Often the first signs of paralysis were accompanied by vomiting and a noticeably foul breath. Convulsions usually began not long after the

appearance of the paralysis. If, however, vitamin B in any of the forms above mentioned, was given after the paralysis had appeared but before it was too late, the polyneuritis was cured by the vitamin in these mammalian experiments, as in the earlier experiments with pigeons and fowls. Figures 3 and 4 from photographs published by Cowgill (1921 b) show the same dog before and after treatment with tomato juice.

Two of the dogs described by Cowgill "showed paralytic symptoms and a slight incoordination of gait; when they exerted themselves to leave their cages, they were seized with spasms and suddenly died. Heart failure was considered to be the cause of death." Here as elsewhere one is struck with the resemblance between the symptoms shown by the dogs deprived of vitamin B and those observed in human beriberi. Continuing Cowgill's description:

"Examination of the leg muscles in those animals which showed paralytic symptoms always revealed a condition of tonic spasticity; the loss of motion in the limb seemed to be due, therefore, not to a degenerative lesion involving the cells of the central nervous system, but rather to the presence of some toxic substance. The opisthotonic position which the body assumes during a convulsion and the hypersensitivity of the entire nervous system at such a time recall to mind the symptoms of strychnine poisoning. The tonic spasticity of the limb muscles disappears when the animal is anesthetized by ether, another fact which is in accord with the idea that a toxic substance is responsible for the symptoms which occur in response to a deficiency of vitamin B."

Cowgill agrees with Karr that the changes in body weight of the animals follow the food intake, so that the loss of weight is not a symptom characteristic of vitamin deficiency as against loss of appetite from other causes, or simple starvation.

The earlier finding of Hopkins (1912) upon rats, that an adequate supply of vitamin in the food results in a more economical use of energy in metabolism, has been confirmed recently by Renshaw (1921) in carefully planned studies. Mice which had been brought to approximate weight equilibrium on a diet of casein, starch, lard, butter, salts, and 1 per cent of yeast gained appreciably in weight when continued for approximately the same time on a ration differing from the first only in the

substitution of an additional amount of yeast (2 or 4 per cent) for its isodynamic equivalent of starch and casein, the vitamin in the yeast presumably effecting a better utilization of the food. In this work no attempt was made to distinguish between the various sources of energy in the ration employed. Funk and Dubin (1920) have reported a much higher percentage increase in weight in rats on a protein-rich than a carbohydrate-rich diet containing 3 cubic centimeters of autolyzed yeast as the source of vitamin B. When the amount of yeast was increased in the carbohydrate-rich rations increased growth took place. This was thought to indicate that a high carbohydrate diet requires more vitamin than a high protein diet.

This theory of a connection between vitamin B and carbohydrate metabolism, first suggested through the apparent relation between beriberi and foods rich in carbohydrate such as polished rice, was emphasized in much of the early work on vitamin B (Funk, 1914 c; Funk and von Schönborn, 1914) and has received further evidence in its support in a brief contribution by Findlay (1921), who reports that in pigeons suffering from beriberi he has found a reduction in the glyoxalase content of the liver by more than half that of control birds. The administration of vitamin B caused a definite rise in the content of glyoxalase. That the vitamin does not act strictly as a coenzyme for glyoxalase was shown by its failure to cause increased production of glyoxalase except when acting through the intact cell. These findings are thought to show that in avian beriberi a definite breakdown in carbohydrate metabolism occurs although whether such a breakdown is followed by the production of toxic substances in the tissues is still undetermined.

The mechanism of the stimulating and curative action of vitamin B has not yet been made clear and it is probably not a simple process. Uhlmann (1918) reported an investigation of the physiological effects of a commercial preparation of vitamin B on the secretion of various glands of rabbits and dogs when administered subcutaneously, intravenously or by the mouth. Other vitamin-B-containing extracts were also used. His main conclusion is that the vitamin acts in a similar manner to pilocarpine, that it incites secretion in the glands of the digestive tract, produces muscular tonus, tends to lower the heart action, dilates the vascular tissues and produces lowered blood pressure,

and that it stimulates the cerebral and vagus nerves. He advances the theory that the vitamin acts as a catalyser in stimulating the endocrine glands.

Dutcher (1918) published evidence that avian polyneuritis is accompanied by a lowering of the catalase activity of the liver, kidney, pancreas, heart, breast muscles, lungs and blood; that shortly after the administration of vitamin extract the catalase content returns to normal and further that pigeons which are relatively immune to polyneuritis possess tissues even under conditions of inanition which are practically normal in their catalase content. On arranging pigeon tissues in order of their catalase content the order is also that of their metabolic activity and of their content of vitamin B. The conclusion drawn at this time was that "it is probable that polyneuritis is accompanied by incomplete or partial oxidation, with the accumulation in the tissues of products of incomplete oxidation. . . . It is also probable that water-soluble vitamins function directly or indirectly in the stimulation of oxidative processes, thereby clearing the tissues of toxic materials." In a later paper from the same laboratory (Dutcher and Collatz, 1918) evidence is presented that the vitamin does not act as a direct activator of catalase but instead stimulates the organism to greater catalase production. With Uhlmann's work in mind Dutcher later (1919, 1920) fed polyneuritic pigeons the stimulants thyroxin, dried thyroid, pilocarpine hydrochloride, and tethelin. While all of these substances apparently produced relief in acute cases of avian polyneuritis, in none of the cases was the response as rapid as when vitamins were fed. The hypothesis is tentatively suggested that "the activity of the organs of internal secretion is dependent upon the stimulatory action of the vitamin. Whether this is in the nature of a nerve stimulant, nuclear nutrient, or the chemical nucleus of a hormone is, of course, a matter of speculation."

Attempts have been made to connect or possibly to identify vitamin B with hormones. Voegtlin and Myers (1919) in a comparative study of the influence on secretion and bile flow obtained evidence which together with the similarity in chemical and physical properties of the two substances led them to suggest the possible identity of the antineuritic vitamin and secretin. The validity of their results and conclusions has since been

questioned by Anrep and Drummond (1921), by Cowgill (1921) and by Downs and Eddy (1921) who have been unable to substantiate their findings.

In a study of the pharmacological action of vitamins A and B reported later by Verzár and Bögel (1920) in which both water and alcohol extracts of wheat embryo were used as sources of vitamin B the results obtained were thought to be not particularly conclusive. While the alcohol extract of vitamin B gave a marked vaso constriction in frog and rabbit preparations, this was not obtained with the water extract, thus indicating that the vaso constricting substance is not identical with vitamin B which is soluble in both alcohol and water, or that the results were complicated by other substances present.

Van Leeuwen and Verzár (1921) report that the reaction of polyneuritic fowls and cats to adrenaline, histamine, and choline as determined by the action on blood and to atropine as determined by inhibition of vagus stimulation, and the reactions of isolated intestine and esophagus of these animals to pilocarpine, atropine, histamine, and choline do not differ materially from similar reactions of normal animals. This is thought to afford sufficient proof that the decreased activity of smooth muscle in polyneuritis is caused by a lack of normal stimulating chemical agents and not by a decrease in sensitiveness of the muscle.

Cowgill and Mendel (1921) have critically reviewed much of the work above mentioned in introducing their own investigation of vitamin B in relation to the secretory function of glands. In their own experiments Cowgill and Mendel used different preparations of B vitamin and tested them with reference to possible influence upon the secretory activity of the pancreas, liver and salivary glands. They summarize their findings as follows:

"A number of solutions, such as extracts of rice polishings, wheat embryo, navy bean and yeast, were shown to contain vitamin B by tests upon polyneuritic animals (dogs and pigeons).

"These solutions were then tested for their possible action on the secretory function of the pancreas, liver and salivary glands. The effect of the products on the rate of flow of pancreatic juice and bile was noted in anesthetized dogs, in which the pylorus was ligated to prevent secretion due to discharge of acid chyme from the stomach, and the discharge of gall bladder bile was

prevented by ligation of the cystic duct. Fresh secretin solutions prepared by the usual method were injected for comparison. Each product was also tested for its possible action on the secretory function of the salivary glands of anesthetized dogs in which the ducts from the submaxillary and sublingual glands were cannulized. Stimulation of the chorda tympani nerve and the injection of pilocarpine served as control procedures in such experiments. The effect of the products on secretory glands was noted in dogs which had subsisted on a normal mixed diet and dogs which had been fed a diet free from vitamin B.

"All of these products demonstrated to contain vitamin B were without any noticeable effect on the rate of flow of pancreatic juice, bile and saliva.

"The intestinal mucosae from eight polyneuritic dogs were examined and found to contain secretin.

"There is no direct relation between vitamin B and the secretory function of the pancreas, liver and salivary glands. The hypothesis that vitamin B functions to stimulate these glands to secretory activity is not supported by the experimental results obtained in this investigation."

Vitamin B in relation to reproduction. One of the findings emphasized by McCarrison was the constant and pronounced atrophy of the testes in males and similar but less marked atrophy of the ovary in females among animals which had been confined to diets lacking vitamin B. Parallel effects in human subjects would lead, he points out, to sterility in males and amenorrhea and sterility in females. Consistently with this Drummond reports that confinement to diet lacking vitamin B soon rendered male rats sterile, and Blunt and Wang report amenorrhea to be common among beriberi women. Too much emphasis should not be laid upon this latter observation alone since beriberi occurs chiefly as the result of too great prominence of polished rice in the diet, and such a diet would be apt to be more or less deficient in vitamin A as well as vitamin B. The same was true of some of the diets used by McCarrison, and as will be shown later, the A vitamin is certainly an important factor in reproduction and is needed in larger quantities for successful reproduction and lactation than for growth and the maintenance of apparent good health.

It is, however, at least suggested by the work of McCarrison

and of Drummond, as well as by some unpublished observations upon breeding rats made by one of us, that vitamin B may be an important factor in reproduction and that successful reproduction and lactation may demand a higher concentration of vitamin B in the diet than is needed for the maintenance of health or even for the support of normal growth.

Vitamin B in the nutrition of bacteria, yeasts, molds and higher plants.

As early as 1901, Wildiers of the University of Louvain reported that if a normal artificial culture medium is inoculated with only a very small amount of yeast the yeast does not grow, while if the same amount be put into sterilized beer wort, or if a larger amount of the yeast suspension be used for inoculating the artificial medium, good growth results. The effect of using the larger inoculum was thought to be due to the presence in all growing yeast cultures, and in beer wort but not artificial media, of a substance essential to the growth of yeast. This substance was said to be soluble in water and 80 per cent alcohol but insoluble in absolute alcohol and ether, dialyzable through porous clay, not precipitable by lead acetate, nor to any extent by phosphotungstic acid or phosphomolybdic acid, silver nitrate in neutral or ammoniacal solution, or mercuric chloride; stable when boiled for one-half hour with 5 per cent sulfuric acid solution but destroyed by boiling with 20 per cent sulfuric acid and to a greater or less extent when boiled with sodium hydroxide in 1 or 5 per cent concentration. To this unknown substance Wildiers gave the tentative name "bios," expressing the desire in so doing that this name would soon give place to a chemical name.

While arousing some attention at the time (Amand, 1903; Devlov, 1906), this contribution of Wildiers seems to have escaped general notice until Funk and others found in yeast a rich source of water-soluble vitamin and the idea naturally suggested itself that the growth of yeast might be made a convenient means of testing for the presence of this vitamin. While this has resulted in an awakened interest in the "bios" problem, the results of renewed investigations along the line of Wildiers have not led to its solution.

Wildiers in concluding his paper referred to above, stated that all varieties of yeast contained this unknown bios. Since then, other fungi and bacteria have been reported to require for their growth something akin to the bios or to water-soluble vitamin. Linossier (1919) reported that *Oidium lactis*, though capable of developing on media exclusively composed of mineral substances and a simple carbohydrate-forming food such as alcohol, acetic acid, glycerol or glucose, is sensitive to the action of vitamins. He is of the opinion that the need of the organism for vitamins is proportional to decrease in vitality, the organism being incapable after a certain point of developing in a medium containing no vitamins. In full activity, however, the organism seems to have the power of existing without vitamins or of synthesizing them. Lumière (1902d, 1921) on the other hand takes the view that fungi and even the higher plants are capable of growing in chemically defined media and even in appropriately chosen solutions which are purely inorganic. Goy (1921) in a study of the food requirements of a number of unicellular organisms including *Saccharomyces cerevisiæ, Mucor mucedo,* and *Penicillium glaucum* found that the proliferation of these lower vegetative forms was remarkably stimulated by the addition of a small amount of the medium in which had formerly been grown either an identical or different species. An organic substance extracted in a crystallizable form from a culture of Mucor by ether is said to differ from the hitherto recognized vitamins in certain

properties, particularly in that it was more active at higher temperatures, but to possess such characteristics as to suggest its being a growth-promoting substance not indispensable to the life of unicellular organisms but singularly efficacious for their proliferation.

This idea has also been brought out recently by Ide (1921) who in an attempt to interpret the conclusion of Wildiers states that: "There are two kinds of proliferation of yeast, one very slow without bios and one fast with bios. Between the two there is such an obvious difference that the distinction could not be overlooked by anyone who has seen it." Willaman (1920) reported the results of an extensive study of the vitamin requirements of *Sclerotinia cinerea,* the brown rot fungus of peaches and plums. He found that while *S. cinerea* can not grow on a medium composed of sucrose, salts and asparagin it grows readily on such a medium when small amounts of plant decoctions, particularly of the fruits of plums and peaches are added. The factor furnished by such decoctions was found to resemble vitamin B in occurrence and properties. While some of the materials tested promoted growth and others growth and reproduction, it is considered that only one vitamin is concerned. "It is very probable that reproduction in Sclerotinia is simply a different manifestation of the same activities as characterize vegetation. The single activity that is apparently most dependent on a vitamin supply is respiration. Respiration is common to all the materials which have yielded the vitamin and the degree of metabolic and hence respiratory activity in these materials is proportional to the activity of the vitamin prepared from them. Thus the evidence is accumulating in favor of the view that there is a close connection between respiration in a cell and its vitamin content and also its vitamin requirement. Just what cells in the plant world can synthesize this vitamin is still an open question. The Sclerotinia vitamin is possibly identical with the water-soluble B of the higher animals and since the latter cannot synthesize this vitamin it becomes an important point to know which plant organs can."

That vitamins of the water-soluble type are essential to the growth of bacteria, both pathogenic and non-pathogenic, seems to be becoming quite definitely established. Lloyd (1916) has presented evidence that the primary cultivation of the meningococcus in vitro is possible only in the presence of certain accessory growth factors present in blood serum, milk and other animal fluids and probably also in vegetable tissues. It was noted in this study that the amount of vitamin required to stimulate the growth of the organism in vitro was inversely proportional to the amount of amino acid present in the medium and that given an abundant supply of amino acid in the medium the organism on isolation from the body became increasingly independent of the vitamin content of the medium, thus suggesting that the function of the vitamin in this particular case is to increase the velocity of the proteolytic metabolism of the meningococcus. Pacini and Russell (1918) concluded that the typhoid bacillus in growing produces vitamins. Kligler (1919) found that the growth of the meningococcus, pneumococcus, streptococcus, *Bacillus diphtheriæ, B. pertussis* and *B. influenzæ* was favorably influenced by the addition of animal tissues and secretions which contain water-soluble vitamin.

McLeod and Wyon (1921) approached the question of the importance of vitamins in promoting bacterial growth first by studying the effect upon the growth of *Staphylococcus aureus* of materials containing vitamin B. They found that in general substances known to contain vitamin B were more potent than those not containing it, but that in certain properties the staphylococcus stimulant differed from vitamin B. These investigators then attempted to determine the value of the growth-promoting property of fresh blood or serum for organisms like the pneumococcus and meningococcus and came to the conclusion that the growth-promoting property of blood for these bacteria must be different in nature from

the known vitamins. They may have failed fully to realize the possibility pointed out by Davis (1917) and recently developed independently by several investigators that in the growth of hemophilic bacilli at least two growth-promoting substances are required, one of the nature of vitamin B and the other of greater heat stability and originally thought to be identified only with the iron-containing pigments of the blood. Davis (1921) from observations on the growth of Pfeiffer's bacillus corroborates his earlier observations and suggests that the heat-labile substance may somehow control or make available the iron in the pigmented portion of the hemoglobin molecule and in this connection suggests the question: Do vitamins or vitamin-like substances influence or to some degree control the metabolism of other elements in the body such as phosphorus, iodine and calcium? Fildes (1921) suggests on the other hand that the relation of the blood pigment in the culture medium to the growth of *B. influenzæ* is rather of the nature of a catalyst which accelerates the transfer of oxygen from the medium to the bacillus. The more copious growth of the bacillus on blood altered by heat or digestion is thought to be due to the fact that in the changed blood the pigment is in the form of hematin and in the unchanged of hemoglobin and methemoglobin. The latter having a stronger affinity for oxygen tends to divert the oxygen from the bacillus which consequently fails to grow. Olsen (1920) likewise suggests that the function of hemoglobin and related compounds is one of catalysis.

A recent series of studies by Thjötta (1921) and Thjötta and Avery (1921, 1921a) has thrown considerable light on the relation of vitamins to the growth of *B. influenzæ* and incidentally to bacterial nutrition in general. They obtained evidence not only that the hemophilic bacteria, of which *B. influenzæ* serves as a type, require for their growth two distinct and separable substances both of which are present in blood and neither of which alone suffices, but further showed that both of these substances can be obtained from other sources than blood. Extracts of yeast, tomatoes, green peas, and beans in dilutions as high as 1:10,000 sufficed to initiate growth of *B. influenzæ* (when seeded from blood media) and to maintain growth for one or two transfers but not more. The failure of continued growth of *B. influenzæ* in a simple medium enriched by one of these extracts was attributed to the exhaustion of the second heat-stable growth factor carried over from the original blood culture. A further study of the nature of this X substance in the blood showed it to be active in a much higher dilution than the V or vitamin heat-labile factor. It was found to resist autoclaving at 120° for 45 minutes and to be still active in blood charcoal which reacts positively to the benzidine test. This suggested the possibility that the benzidine reaction might serve as an indicator of the presence of the X factor in other substances and following this suggestion the potato, which reacts to the benzidine test, was found to furnish both the V and the X factors. *Bacillus influenzæ* heretofore considered as an obligate hemophile was found to grow luxuriantly in plain buffer solutions of sodium and potassium phosphate ($pH = 7.5$) containing unheated potato. These results are thought to be of wide significance in showing that bacteria as well as animals require growth-accessory substances and that the "hemophilic" property of this group of organisms has been based on a lack of knowledge of their essential nutritional needs. Rivers (1921) has reported the presence in eggs of the autoclave-stable factor in blood necessary for the growth of *B. influenzæ*. Rivers and Poole (1921) are of the opinion that the phenomenon of augmented growth of influenza bacilli in symbiosis with other bacteria under certain conditions is due to the synthesis of the autoclave-labile factor and Williams and Povitzky (1921) have presented evidence that both the X and V factors are present in certain living organisms but not in dead cultures of the same stimulating organisms or their extracts.

Thus considerable evidence is at hand that yeasts, molds, and bacteria require for their growth certain water-soluble substances resembling in many ways vitamin B and that in some cases the living organism apparently has the power to synthesize its own vitamins or in other words to be stimulated by the products of its own growth. Meantime largely through the work of Bottomley and Mockeridge the theory has been developed that green plants, which in their turn furnish vitamins to animal life, require for their successful growth minute quantities of organic substances, which have been termed auximones by Bottomley (1914) who has found that the auximones are produced very largely during the bacterial decomposition of peat (and humus?) by aerobic organisms. Mockeridge (1917) working in the same laboratory, has shown that the auximones stimulate not only plant growth but also the activity of the soil organisms from which they originated. Bottomley (1919) found that the products of the nitrogen-fixing bacteria *Azotobacter chroococcum* and *Bacillus radicocola* grown on pure synthetic media, can function as growth promoting substances for the higher plants.

Working on the supposition that the value of ordinary organic manures may be due partially to the presence of growth-promoting substances which have been set free by bacterial action, Mockeridge (1920) prepared water extracts of fresh and well rotted stable manures, leaf mold and well manured garden soils and tested their growth-promoting power for the common duckweed *(Lemna major)* when added in certain proportions to inorganic nutrient solutions. The increase in growth following upon the addition of these materials to the culture solution was greater than could be attributed to the "purely nutritive" value of the materials added and varied directly with the extent of the bacterial decomposition of the material tested. From microscopic examination she concluded that the auximones have a marked influence on the size and contents of the cell and especially of the nucleus. Examination of materials which furnished the auximone preparations showed them to contain appreciable quantities of nucleic acid or its cleavage products. Mockeridge thus suggests a relationship of the water-soluble growth-promoting substance to the nuclear metabolism of the plant which is of special interest in connection with the work of Williams and of McCarrison connecting vitamin B with nucleic acid derivatives and with the metabolism of the nucleus in animal nutrition.

Detection and Measurement of Vitamin B.

In the past the most common means for the detection of vitamin B has been the classical method by the use of which the existence of such a substance was discovered, namely, the cure or relief of the neuritis which develops in pigeons or fowls when they are fed upon polished rice as a sole food, or upon other diet similarly deficient in the B vitamin. This method, however, has been found in the experience of many if not all of the investigators who have used it, to give rather irregular results. There is no satisfactory way of determining the degree of polyneuritis from the symptoms or of judging the exact time at which the curative experiment should be performed. Naturally it must be expected that a vitamin preparation or a vitamin-

containing food which shows good antineuritic effects upon one bird may fail to cure a second because in the second case the disease had progressed so far as to preclude recovery. Still greater difficulty arises from the fact that birds which have developed typical polyneuritis on vitamin-free or vitamin-poor diet sometimes recover spontaneously without any change in their food or the administration of vitamin in any form. This being the case it necessarily follows that neither the positive nor the negative results obtained in such "curative" experiments can be entirely conclusive in any individual case. Safe conclusions can be drawn only from a preponderance of the evidence resulting from numerous experiments. Obviously it will be still more difficult to draw from such curative experiments any quantitative conclusions as to the relative concentration of the vitamin in different materials or in the same material before and after treatment.

Yet as we have seen earlier in this chapter, it is only through quantitative comparisons of the antineuritic and growth-promoting properties of foods that we can hope to reach a solution of the apparently qualitative question whether the antineuritic and water-soluble growth-promoting vitamin are the same substance.

Obviously too, only quantitative measurements can determine the relative values of different foods as sources of the vitamin, or can show at all conclusively whether a loss of vitamin occurs in, for example, the cooking, canning, or storage of a food.

Undoubtedly the best method of determining the relative amount of any vitamin in a food is to make the food to be tested the sole source of this particular vitamin in a dietary so constituted as to be adequate in all other respects, and then to determine the minimum quantity or proportion of the food which will just protect a standard test animal from the characteristic deficiency disease, or enable it to maintain a normal rate of growth.

With a well chosen basal dietary fed to an animal of suitable species and of standard age and weight, the amount of the food under investigation which will be needed for protection from deficiency disease or for support of normal growth will be inversely proportional to the concentration of vitamin in the food, provided no disturbing factor is allowed to enter into the test.

This minimum allowance of the food in question may be determined either in terms of the weight required per day when the test food is fed separately from the rest of the diet, or of the proportion in which the test food must enter into the food mixture in order to make the ration adequate. In either case the total food intake should be determined and the adequacy of the diet in respect to all other nutritive requirements should be assured. Obviously in either case the determination of the "minimum protective dose" of the food or of the minimum allowance for support of normal growth is likely to require a series of experiments in which the quantity or proportion of the test food is systematically varied while all other conditions are held constant. Such a series of quantitative experiments is naturally much more exacting than the few tests which would suffice to demonstrate the presence of the vitamin qualitatively, and it is not surprising that in the years which have been so largely spent in demonstrating the actual existence of vitamins and learning something of their occurrence in plant and animal organisms, the majority of investigators should have felt it more interesting to survey the general field qualitatively than to spend the large amount of time and labor necessary to make quantitative comparisons. Now, however, the qualitative survey has served its purpose and henceforth no investigator should be satisfied with anything less than at least approximately quantitative work.

The importance of accurate and quantitative measurements in experimental work upon nutrition with special reference to vitamin problems has been well emphasized by Chick and Hume (1919). While their short paper on this subject should be read in full by all who are interested in this field of investigation, certain sections may well be quoted here.

According to Chick and Hume, "Since the general conception of accessory food factors (or vitamins) was brought forward a constant succession of researches has been made to assign values to various foodstuffs as antineuritic, as antiscorbutic, or as growth-promoting materials respectively, and further to determine whether these values are lessened or destroyed by heating, drying, or other methods of preservation. In most of this work one fundamental consideration—namely that of *quantity*, has been to a large extent overlooked in the methods adopted for

experiment, and the conclusions drawn have been untrustworthy to a corresponding degree.

"In comparing the values of a series of foodstuffs as regards their value in content of some accessory food factors, it is obvious that the first step necessary is to determine in each case the minimum daily dose which will maintain health in the experimental animal and to institute comparison between these amounts * * *

"Similarly, in investigating the effect of heat, drying, or any other method of preservation upon the accessory factors contained in any foodstuff, it is necessary to determine the minimum daily dose (1) of the original untreated material and (2) of the same material after heating, drying, etc., which will protect the same experimental animal under similar conditions of experiment. Then only is it possible to state to what extent, if any, the foodstuff has suffered deterioration as the result of the treatment.

"Neglect of this simple and fundamental, but admittedly tedious procedure is evident in many papers recently published upon the influence of various treatments upon the antineuritic and antiscorbutic and other properties of certain foodstuffs and the results obtained are in consequence vague, and may even be erroneous. It is clear, for example, that if a foodstuff is rich in any particular accessory factor and is fed to an animal in large excess of the amount actually required, any destruction of the factor that may take place during heating cannot be assessed and may even remain undetected if the heated material is fed in similar large amount. It is absolutely necessary to determine the minimum required in both cases, and to institute a comparison between these two results before any satisfactory conclusion on this point can be drawn."

Attempts to measure the vitamin B content of any given material ordinarily make use of one of the three following methods.

(1) Determination of the amount necessary to cure or preferably to prevent polyneuritis in pigeons.

(2) Determination of the amount or proportion needed in the food of young rats to support a normal rate of growth.

(3) Measurement of the influence of the material upon the growth or activity of yeast.

The first is plainly a measure of antineuritic vitamin, and the second of water-soluble growth-promoting vitamin or "water-soluble B." So long as there is doubt as to whether these are the same substance there must be a corresponding degree of doubt as to whether the values obtained by experiments upon birds and upon rats can properly be treated as interchangeable.

Still more doubtful is it if the yeast method measures the same thing as is measured by the feeding experiments either with birds or with mammals. While we do not feel that the yeast method in any form in which it has yet been described can be accepted as a trustworthy means of measuring vitamin content, yet because of the extent to which this method has recently been used, it seems advisable to include here a brief account of its origin and status.

As previously noted, Wildiers as early as 1901 reported that if a normal artificial culture medium is inoculated with only a very small amount of yeast, the yeast does not grow, whereas, if the same amount be put into sterilized beer wort, or if a larger amount of the yeast suspension be used for inoculating the artificial medium, good growth results. The effect of using the larger inoculation was said to be due to the presence in all growing yeast cultures (and in beer wort but not artificial media) of a substance essential to the growth of the yeast which pending chemical identification Wildiers called "bios." A concise account of Wildiers' observations in this connection is given by Bayliss (*Principles of General Physiology,* 1915, p. 260). Wildiers' work seems to have been almost entirely ignored until Funk and others found in yeast a rich source of water-soluble vitamin. The idea then naturally suggested itself that growth of yeast might be made a convenient means of testing for the presence of the vitamin, and several methods based upon this idea have been proposed. Bachmann (1919), R. J. Williams (1919, 1920), Eddy and Stevenson (1920), Funk and Dubin (1920), and Swoboda (1920) are among those who have regarded the yeast method highly as a means of testing for the presence and even for the relative concentration of the water-soluble vitamin. Funk (1920) finds his yeast vitamin prepared and tested as antineuritic in 1912 to be active in stimulating yeast growth and has recently used the yeast method in a reinvestigation of the isolation of vitamin B. On the other hand, Emmett

and Stockholm (1920) conclude that the substance which promotes the growth of yeast is probably not the same as the antineuritic vitamin and is also possibly different from water-soluble B. "Since the amount of yeast growth stimulus, expressed in terms of yeast cell units per gram of substance does not appear necessarily to vary directly (in terms of potency) with the antineuritic and water-soluble B vitamins, this yeast method should not be employed quantitatively with too much definiteness until further study is made."

Souza and McCollum (1920) criticise the yeast method on the ground that stimulation of yeast growth may be due to many other substances and is therefore not a reliable indication of the presence of vitamin. "The series of observations which we have presented above seem to us to form a conclusive demonstration that the use of yeast as a test organism for determining the presence or absence of the antineuritic dietary factor is complicated by so many disturbing factors as to make it of little if any value." Fulmer, Nelson and Sherwood (1921) emphasize the importance of quantitative variations of the substances employed in making up the culture medium and hold that when each of the known constituents is used in optimum proportion the growth of the yeast is so active that it cannot be accelerated further by the addition of vitamin.

Very recently Eddy, Heft, Stevenson and Johnson (1921), who have been among the active workers employing the yeast method in vitamin investigation, have presented extensive data which as a whole seem to indicate quite clearly that, while vitamin B may stimulate yeast growth, yet the conditions influencing the rate of growth of yeast must be much more definitely determined before it can be assumed that in any given case the rate of growth of yeast (even as compared with careful controls) is a quantitative measure of the vitamin added to its culture medium. Doubtless in some cases the yeast method has led to correct conclusions, but it seems equally probable that in other cases the stimulation of yeast growth has been largely due to other substances in the food or extract which was being tested for vitamin. The possible presence of substances inhibitory to yeast growth also constitutes a serious complication. Yet it appears not impossible that, under a sufficiently rigorous system of control tests, accelerated growth of yeast may in some cases

afford a useful indication of the probable presence of vitamin B. In our opinion a still more thorough study of media and of the comparative results obtained by applying yeast tests and animal feeding experiments to identical materials must precede any conclusive interpretation of results obtained in the study of vitamin problems by the yeast method.

Returning now to the detection and measurement of vitamin B by animal feeding experiments, it is, of course, always to be kept in mind that the antineuritic vitamin and the water-soluble growth-promoting vitamin B may or may not be the same substance. Hence if antineuritic properties purely are to be studied, pigeons are probably preferable to rats as test animals and the individual variability of the pigeons should be offset by the use of larger numbers of birds.

Recently Simonnet (1920) has proposed the use of the following ration for the study of polyneuritis in pigeons:

Meat residue prepared by double extraction with boiling alcohol and ether 11 grams, Osborne and Mendel's salt mixture 4, pulverized agar 5, peanut oil heated for 3 hours at 130° C. 5, cellulose 5, butter melted, decanted, and filtered at a temperature below 40° 10, and potato starch 60 grams. These ingredients are intimately mixed with 80 per cent of their weight of water and made into pellets, which are introduced into the crops of the experimental pigeons in two daily doses in amounts equal to one-fifth the weight of the bird. The pigeons are kept in very large cages to allow considerable freedom.

On the above diet the pigeons are said to maintain their weight but to develop a spasmodic form of avian polyneuritis (unaccompanied by paralytic and atrophic symptoms, in the development of which inanition and multiple deficiencies are considered to play the important rôle). The disease appears generally in about 30 days, and is usually fatal in about 4 days if no treatment is given. The administration of dry or autolyzed yeast brings about prompt recovery. By supplementing the deficient ration by the administration of 0.5 gram of dried yeast daily, the pigeons can be maintained in excellent health for more than 7 months.

The use of a diet thus planned to provide for all other nutritive requirements might well help in minimizing the variability of results which has so handicapped the study of relative

amounts of vitamin B in foods when pigeons on polished rice diet have been used as experimental animals.

If vitamin B is interpreted as meaning primarily the growth-promoting substance to which the designation B has usually been applied, then the best animal for use in feeding experiments to detect and measure the vitamin B is the rat, since the nutritive requirements and rate of growth of the rat have been so exten-

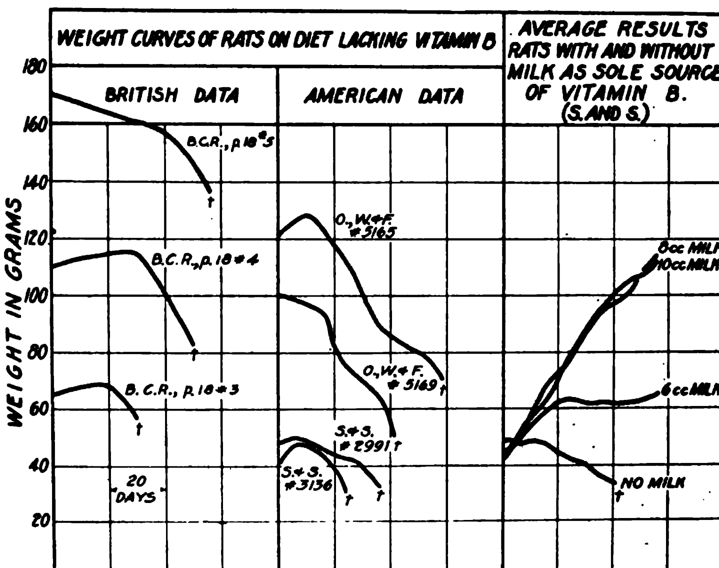


FIG. 5.—Weight curves of rats on diets adequate in other respects but lacking vitamin B. British data from Report of Medical Research Committee of Great Britain, by permission. American data from Osborne, Wakeman and Ferry (by courtesy of the *Journal of Biological Chemistry*) and from Sherman and Spohn.

The curves in the right-hand section of the figure show the results of feeding rats of the same age upon the same diet without vitamin B, and with measured amounts of milk as a sole source of this vitamin.

sively studied. Fig. 5 shows weight curves of rats used in such experiments upon vitamin B.

The three curves in the left hand column of the figure are from the British Committee Report (Hopkins, Chick, Drummond, Harden and Mellanby, 1919), and illustrate the fact that the older and larger the animal when placed upon diet devoid of vitamin B, the longer it may be expected to live.

This is also illustrated by the four curves in the middle column, two of which are from the work of Osborne, Wakeman

and Ferry (1919) and two from unpublished experiments by Sherman and Spohn.

In the right hand column are shown average results of experiments made by Sherman and Spohn to determine the amount of milk needed to furnish the B vitamin required to support normal growth in the young rat. Starting with rats four weeks of age and weighing 40 to 50 grams, it will be seen that on diet without vitamin B the duration of life was about six weeks during which time about one third of the initial body weight was lost. With 6 cubic centimeters of milk daily as sole source of vitamin B, growth occurred but at a subnormal rate. With 8 cubic centimeters of milk the growth rate was practically the normal average and was not accelerated by increasing the daily allowance of milk to 10 cubic centimeters. Hence it is concluded that in these experiments the amount of milk required to furnish the necessary amount of B vitamin for support of normal growth in the young rat was 8 cubic centimeters per rat per day. The milk here used was prepared by mixing 9 parts water with 1 part dried skimmed milk, so that a more precise statement of the result would be that the amount of B vitamin required was furnished by 0.8 gram of dried skimmed milk, equivalent to 8 cubic centimeters of milk in the natural fluid state.

The method of measurement of B vitamin content by finding the amount of the food in question required for support of growth in rats has been much used by Osborne and Mendel with results which will be summarized in the following section. In some cases they have recorded the quantities sufficing for maintenance instead of for growth. We have not found any series of experiments designed primarily for the determination of the quantitative relation between the requirements for growth and for maintenance. Some observations on this point by Sherman and Spohn indicate that for rats placed on experimental diets at four weeks of age, continued growth at approximately the normal rate requires about twice as much vitamin B as does maintenance of weight with growth suspended. The relation should be determined more definitely and for different ages.

Recently Dunham (1921) has described a method for the quantitative comparison of the vitamin B content of vegetables as follows: Albino rats from 4 to 5 weeks old were fed *ad libitum* a basal ration of casein 20, starch 61, butter fat 15, and salt

mixture 4 per cent until growth had ceased and in some cases a slight decrease in weight had occurred. The vegetable to be tested, air-dried and pulverized, was then fed in decreasing amounts apart from the daily ration until the weight became constant at some point between 75 and 100 grams, when the rat was 100 to 130 days old. The feeding of the basal ration plus this amount of vitamin was continued for 30 days, during which time the weight should remain constant with an allowable variation of ± 4 per cent. At the end of this period 500 milligrams daily of dried yeast was added to the diet in place of the substance tested. Immediate restoration of growth would indicate that the lack of growth was due to insufficiency of vitamin B.

Distribution of Vitamin B in the Body and in Food Materials.

This vitamin has been shown to occur in the most varied parts of plants and animals. A list of the materials in which it has been reported is given in the accompanying table. When, however, the study of the vitamin content of foods is approached from the quantitative point of view, and it is also remembered that the question still remains open as to whether the antineuritic and water-soluble growth-promoting vitamins are the same, it is apparent that much of what can now be said must necessarily be both vague and tentative.

Distribution in the body. Probably the concensus of opinion in regard to this particular phase of beriberi as a deficiency disease is to the effect that the body contains a larger or smaller amount of the antineuritic vitamin, a part of which is in the muscles. When the amount furnished by the food is insufficient the vitamin needed for the normal functioning of the nervous system is for a time supplied at the expense of the muscles so that more or less wasting of the muscles usually occurs with or before the appearance of the nerve symptoms. This accords both with the emaciation ordinarily observed (unless masked by edema) in human beriberi and with the appearance of creatinuria in Drummond's animals when kept on food lacking the B vitamin.

Cooper (1914) recorded the following amounts of animal products as necessary to prevent polyneuritis:

	In terms of natural food material.	*In terms of dry weight.*
Ox voluntary muscle (beef)	20 grams	5.0 grams
Ox cardiac muscle (beef heart)	5 "	1.7 "
Ox cerebrum	6 "	1.2 "
Ox cerebellum	12 "	2.4 "
Ox liver	3 "	0.9 "
Cows' milk	35 "	3.5 "
Egg yolk	3 "	1.5 "

By feeding animal tissues as the sole source of water-soluble vitamin for the support of growth in rats, Osborne and Mendel (1918a) showed that skeletal muscle (ordinary meat) is poor in this vitamin, while heart muscle, liver, kidney, and brain contain it in larger proportion.

Eddy (1916) had also shown the presence of notable amounts of water-soluble vitamin in pancreas tissue.

McCollum (1918) speaks of a relatively high vitamin B content in glandular organs. Conversely Funk and Douglas (1914) had reported that with few exceptions the organs of internal secretion are atrophied in pigeons rendered polyneuritic through lack of vitamin B.

Swoboda (1920), using the yeast multiplication method, reports that "the organs of internal secretion which are of developmental importance" are relatively rich in vitamin B. He finds the pituitary and pineal glands to be highest in vitamin content, and most of the other organs of internal secretion—suprarenal, testis, ovaries, and thyroid—to contain about the same amount of vitamin per gram of dried tissue. The concentration in liver and kidney was slightly higher; that in the thymus, pancreas, and lymph glands less. Swoboda emphasizes the prominence of vitamin B in those organs which are important in connection with sex development. This is of interest in connection with Drummond's observation that abundance of vitamin B in the diet is favorable to reproduction in laboratory animals (rats), a conclusion which appears to be confirmed by the records of our own rat colony.

Reported Occurrence of Vitamin B.

Food Material	*Literature Reference*
Alfalfa	McCollum, Simmonds and Pitz 1916a, 1917b; McCollum 1917; Osborne and Mendel 1919, 1919c, 1920a; Steenbock and Gross 1920; Eddy and Stevenson 1920.
Apple	Eddy and Stevenson 1920; Osborne and Mendel 1920d.
Artichokes	Santos 1921.
Avocado (alligator pear)	Santos 1921.
Bamboo shoots	Santos 1921.
Barley	Eijkman 1906; Holst 1907; Cooper 1912, 1914a; Steenbock, Kent and Gross 1918.
Beans	
katjang idjo (*Phaseolus radiatus*)	Grijns 1901; Edie et al 1912.
navy (*Phaseolus vulgaris*)	McCollum, Simmonds and Pitz 1917a; McCollum and Simmonds 1918; Daniels and McClurg 1919; Miller 1920.
soy	Daniels and Nichols 1917; Osborne and Mendel 1917e; Cohen and Mendel 1918; Daniels and McClurg 1919; Johns and Finks 1921.
Beer	Harden and Zilva 1918e.
Beet (root, stem, leaves)	Osborne and Mendel 1919c, 1920a.
Brain	Funk 1912a, 1912b; Cooper 1914; Osborne and Mendel 1918a.
Cabbage	McCollum and Kennedy 1916; McCollum 1917; Daniels and McClurg 1919; Osborne and Mendel 1919, 1919c, 1920a;

Food Material	*Literature Reference*
Cabbage	Shorten and Roy 1919, 1921; Steenbock and Gross 1920; Whipple 1920; Dunham 1921.
Carrots	Sugiura and Benedict 1918; Sugiura 1918; Denton and Kohman 1918; Shorten and Roy 1919, 1921; Steenbock and Gross 1919; Zilva 1920; Miller 1920; Osborne and Mendel 1920a; Eddy and Stevenson 1920; Dunham 1921.
Celery	Eddy and Stevenson 1920.
Clover	McCollum 1917; Osborne and Mendel 1919, 1919c, 1920a; Steenbock and Gross 1920.
Coconut press cake	Johns, Finks and Paul 1919.
Corn	McCollum, Simmonds and Pitz 1916d; Chick and Hume 1917, 1917a; Voegtlin, Lake and Myers 1918; Green 1918a, 1918b; Voegtlin and Myers 1919.
Corn pollen	Dutcher 1918.
Cucumber	Eddy and Stevenson 1920.
Dandelion	Osborne and Mendel, unpublished.
Dasheen	Steenbock and Gross 1919.
Eggplant (sun dried)	Shorten and Roy 1921.
Egg yolk	Eijkman 1911; Cooper 1912, 1914a; McCollum and Davis 1915b; McCollum 1916; Steenbock 1917; Osborne and Mendel 1919.
Egg whole	Chick and Hume 1917b.
Fish	Drummond 1918a;
roe	Chick and Hume 1917b.

Food Material	*Literature Reference*
Flour, wheat	Chick and Hume 1917a, 1917b; Voegtlin, Lake and Myers 1918; Voegtlin and Myers 1919; Osborne and Mendel 1919a.
Glandular organs (See also Pancreas, Thymus, etc.)	Funk and Douglas 1914; McCollum 1918; Swoboda 1920.
Grapefruit	Osborne and Mendel 1920d.
Heart muscle	Cooper 1912, 1914a; Osborne and Mendel 1918a.
Kafir	McCollum, Simmonds and Parsons 1919.
Kidney	Osborne and Mendel 1918a; Swoboda 1920.
Lemon juice	Osborne and Mendel 1920d.
Lettuce	Osborne and Mendel, unpublished.
Lentils	Cooper 1912, 1914a.
Lime juice	Funk 1912a, 1912b.
Liver	Cooper 1912, 1914a; Osborne and Mendel 1917b; Swoboda 1920.
Malt extract	Cooper 1914a.
Meat (See Muscle)	
Meat extract	Osborne and Mendel 1917b.
Milk, whole, dried	Hopkins 1906, 1912; Osborne and Mendel 1911; Andrews 1912; Funk 1912a, 1912b; Gibson 1913a; Hopkins and Neville 1913; Cooper 1914; Gibson and Concepcion 1916; McCollum, Simmonds and Pitz 1916c; Osborne and Mendel 1918c, 1920b; Karr 1920; Johnson 1921.
whole, boiled	Gibson and Concepcion 1916.
whole, condensed	Rosenau 1921.
whole, pasteurized	Johnson 1921.

Food Material	*Literature Reference*
Milk, whole, dried	Osborne and Mendel 1919; Sherman, Rouse, Allen and Woods 1921; Dutcher, Kennedy and Eckles 1920; Rosenau 1921.
skim, dried	McCollum and Davis 1915; Johnson 1921; Johnson and Hooper 1921.
Milk, human	Osborne and Mendel, unpublished.
Millet	Eijkman 1906; McCollum 1917.
Mongo (bean)	Santos 1921.
Muscle	Grijns 1901; Eijkman 1906; Holst 1907; Cooper 1912, 1913a, 1914a; Voegtlin and Lake 1918, 1919.
Nuts	Cooper 1914a; Cajori 1920.
Oats	Eijkman 1906; McCollum and Kennedy 1916; McCollum, Simmonds and Pitz 1917.
Okra	Santos 1921.
Onions	Osborne and Mendel 1919c; Shorten and Roy 1919, 1921; Eddy and Stevenson 1920; Whipple 1920.
Orange	Osborne and Mendel 1920d;
juice	Byfield, Daniels and Loughlin 1920.
inner peel	Osborne and Mendel 1920d.
Pancreas	Eddy 1916, 1917, 1917a; Eddy and Roper 1916, 1917; Swoboda 1920.
Parsley	Osborne and Mendel, unpublished.
Pea	Holst, 1907; Chick and Hume 1917b; McCollum, Simmonds and Parsons 1919a; Dunham 1921.
Peanut	Greig 1918; Daniels and Loughlin 1918; Johns and Finks 1920.

Food Material	*Literature Reference*
Pear	Osborne and Mendel 1920d.
Potato, sweet	Steenbock and Gross 1919.
Potato, white	Eijkman 1906; McCollum and Kennedy 1916; Chick and Hume 1917; McCollum, Simmonds and Parsons 1918a; Osborne and Mendel 1920a; Eddy and Stevenson 1920; Dunham 1921; Shorten and Roy 1921.
Prunes	Osborne and Mendel 1920d.
Radish	Eddy and Stevenson 1920.
Rice (unmilled)	Eijkman 1896-1906; Grijns 1901; Cooper and Funk, 1911; Eijkman 1911; Funk 1911, 1912a, 1912b, 1913c; Chamberlain 1911; Heiser 1911; Chamberlain and Vedder 1912; Suzuki et al 1912; Fraser and Stanton 1911-1915; Vedder and Williams 1913; Wellman, Eustis and Scott 1914; Albert 1915; McCollum and Davis 1915b; Williams and Saleeby 1915; Chick and Hume 1917, 1917a; Jansen 1920a.
Rutabaga	Steenbock and Gross 1919.
Rye	Eijkman 1906; Holst 1907.
Spinach	Osborne and Mendel 1919, 1920a; Shorten and Roy 1919, 1921; Dunham 1921.
Thymus (sweetbread)	Swoboda 1920.
Thyroid	Seaman 1920.
Timothy	Osborne and Mendel 1919, 1919c, 1920a.

Food Material	*Literature Reference*
Togi (sprouted mongo)	Santos 1921.
Tomato	Osborne and Mendel 1919c, 1920a; Eddy and Stevenson 1920; Karr 1920; Cowgill 1921b;
sun dried	Shorten and Roy 1921.
seed press cake	Finks and Johns 1921;
Turnip	Osborne and Mendel 1919c, 1920a; Eddy and Stevenson 1920; Dunham 1921.
Wheat	McCollum and Davis 1915b, 1915d; McCollum and Kennedy 1916; McCollum, Simmonds and Pitz 1916, 1916c; Chick and Hume 1917, 1917a, 1917b; Voegtlin, Lake and Myers 1918; Daniels, Byfield, and Loughlin 1919; Voegtlin and Myers 1919; Osborne and Mendel 1919a; Stammers 1921.
Yeast	Eijkman 1911; Cooper 1912, 1914; Edie et al 1912; Funk 1912b, 1913b, 1913c, 1916; Seidell 1917; Emmett and McKim 1917; Chick and Hume 1917; Drummond 1917; Sugiura and Benedict 1918; Sugiura 1918; Thompson and Mendel 1918; Hawk, Fishback and Bergeim 1919; Osborne and Mendel 1919b; Osborne and Wakeman 1919; Karr 1920; Myers and Voegtlin 1920; Abderhalden 1920; Hawk, Smith and Bergeim 1921; Dunham 1921.

Meat. From the distribution of vitamin B in the animal body, as indicated above, it is clear that ordinary meats are poor in this vitamin, while heart, liver and kidney contain it in

somewhat larger proportion. Quantitative comparisons either of these meats with each other or with other foods, cannot readily be drawn from the data thus far available, but the experiments of Osborne and Mendel make it plain that ordinary meat is not to be depended upon to furnish the water-soluble vitamin needed for growth. With 20 per cent of dry meat in the food mixture given to growing rats there was early and marked failure for lack of vitamin B, the weight curves resembling those obtained when this vitamin was practically entirely absent. On the other hand, rations containing the dried tissue of heart, liver, or brain in the proportions of 19, 22, and 32.5 per cent respectively seemed to furnish enough of the B vitamin for the support of normal growth. The contrast between ordinary meat on the one hand and heart, liver or brain on the other, appears even more strikingly here than in Cooper's comparison of the amounts required to prevent polyneuritis. By comparison with experiments reported by Osborne and Mendel elsewhere it would appear that heart, liver and brain contain vitamin B in proportions somewhat similar to those in which it is found in milk, eggs and whole cereals (all being compared on the basis of solid matter), whereas ordinary muscle meat showed too small an amount to admit of any quantitative comparison.

Chick and Hume report that canned meat is of practically no value as a source of B vitamin.

Eggs. There is probably as high a concentration of vitamin B in the egg, or at least in the egg yolk, as in any part of the body. This is indicated both by the experiments of Cooper upon prevention of polyneuritis and those of Osborne and Mendel upon the support of growth. The latter results have apparently not yet been published in full but are referred to in terms which would imply that the solids of eggs and of milk have about equal proportions of the water-soluble vitamin.

Since even slight deterioration of an egg is so readily perceptible to the senses, it is probable that eggs preserved by any method sufficiently efficient to keep them salable will retain their original vitamins in practically undiminished proportion. Whether the vitamin can withstand all the treatments to which eggs are sometimes subjected in cookery may be more doubtful.

Milk. The mammary gland is believed not to synthesize vitamin but to take it from the blood, or through the blood from

the tissues, of the mother and convey it to the young through the milk. Since the total amount of B vitamin available in the tissues is relatively small, the concentration in which it appears in the milk is largely dependent upon the vitamin content of the mother's food. This doubtless is one reason that different investigators have received different impressions as regards the richness of milk in this vitamin. Another reason is to be found in the observation of Osborne and Mendel (1918c, pp. 539-40) that individual animals vary greatly in their requirements for the water-soluble vitamin; and still another in the differing lengths of experiments and criteria of satisfactory growth. In some cases the experiment has been deemed successful and the food satisfactory when growth at a nearly average rate was maintained for a few weeks, while other investigators, such as Osborne and Mendel, have discussed the question in terms of the requirement for *optimum* growth. If to a basal ration milk is added in increasing proportions, it is probable that the diet, even though adequate before, will be improved by the increased proportion of milk in other respects as well as in its content of water-soluble vitamin, and so the experimenter will be led to the use of more milk than is really necessary to supply the needed amount of vitamin B. From a study of the published work of previous investigators and the results of several unpublished experiments of our own, we judge that milk solids contain about as much of the B vitamin as the solids of eggs and much more than the solids of ordinary meat; and that on the average an amount of milk sufficient to furnish from 10 to 20 per cent of the total solids or total calories of the diet will furnish as much water-soluble vitamin as is needed to make the food mixture adequate for growth.

Osborne and Mendel report (personal communication) that human milk is not richer than cow's milk in vitamin B.

Cereals and Breadstuffs. The whole grains of the cereals are fairly rich in vitamin B and a diet consisting largely of grain products would probably always contain an ample amount of this vitamin were the entire grain used; but the interior of the endosperm contains much less of it than the other parts of the grain and so the more highly refined the mill product the greater the danger that vitamin content may be too greatly reduced.

Since the phosphorus content of the endosperm is also much

lower than that of the whole grain, there exists in the case of the mill products of rice, or wheat, or maize, a rough parallelism between the phosphorus content and the B vitamin content which is sometimes useful in permitting a quick judgment of the character of a product by determining the amount of phosphorus it contains.

In rice there is a high concentration of vitamin B in the embryo, a lower concentration in the bran, and little if any in the pure endosperm. The embryo is small and readily detached from the endosperm in the milling process, while the successive layers between the husk and the endosperm are not removed so readily. Hence any milling of rice is likely to lower greatly its antineuritic value through the loss of the embryo, but rice which is not too highly milled still has some antineuritic value. In pure white polished rice, however, the vitamin content is so far reduced that it is a disputed point whether such rice contains any vitamin B whatever.

In wheat the B vitamin appears to be somewhat less definitely localized than in rice. Commercial wheat embryo has been used by several investigators as a rich source of vitamin; but when Osborne and Mendel fed pure wheat embryo which had been carefully removed from the kernels by hand in the laboratory, its B vitamin content was found lower than would have been expected. Their work suggests that a crumbly layer of endosperm lying next the embryo and which ordinarily accompanies the latter in its commercial separation, may be the part of the wheat kernel which is richest in the B vitamin. Patent flour representing the part of the wheat endosperm poorest in vitamin is not so devoid of it as is polished rice. While white wheat flour is poor in B vitamin as compared with the whole grain, it yet contains enough to be of considerable significance in nutrition.

Osborne and Mendel report (Carnegie Institution Year Book No. 17, p. 305, 1919) that 50 to 60 per cent of patent flour in an otherwise adequate diet furnishes barely sufficient vitamin (B) to promote satisfactory growth in the rat. On the other hand such growth was supported by the vitamin B furnished by 15 to 20 per cent of entire wheat in the diet, or by 2 to 5 per cent of the preparation of commercial wheat embryo which they used.

They add: "So long as the diet of man is sufficiently varied

to include enough vitamin from sources other than the cereals, the milling question need not awaken much concern from the vitamin standpoint; but when the diet is restricted the danger of a shortage of vitamin may become real if a large proportion of sugar, fat, or other products, low in their content of water-soluble vitamin, are included in the diet." Since this is often the case and since we now have reason to believe that an intake of vitamin much more abundant than is absolutely necessary is often advantageous, we believe that the use of whole grain in preference to highly milled products should be encouraged.

Since yeast is rich in B vitamin, the introduction of yeast in the making of bread must raise its vitamin content slightly, but only very slightly because the amount of yeast used is so small. The use of a "lower" grade of flour (less purely the interior of the endosperm) may be expected to increase the vitamin content of the bread much more than does the yeast used for leavening.

Beans, peas, and seeds in general, when eaten entire or approximately so, are apparently all rather similar as sources of B vitamin. McCollum states that with 25 per cent of white beans in the diet as sole source of B vitamin the supply of this was fully adequate. McCollum found 15 per cent of whole wheat to be hardly adequate as sole source, while 35 per cent is more than adequate. Fairly similar results have been obtained with peas, soy beans, barley and doubtless other seeds as well. Steenbock found 20 per cent of barley to be slightly less than fully adequate. Osborne and Mendel (1919) speak of whole wheat, soy beans, dried eggs and milk solids as each being required in proportions twice as large as of dried spinach or eight times as large as of yeast, when serving as sole source of vitamin B. This would imply that whole wheat, soy beans, dried eggs, and milk solids are each from four to six times as rich in B vitamin as the diet as a whole needs to be.

Vegetables and Fruits. The potato was found by McCollum, Simmonds and Parsons (1918a) to be capable of supplying all of the needed B vitamin; but since large proportions of potato were used in all the experiments which they describe, their data do not show how the potato compares with other foods quantitatively in this respect. Osborne and Mendel (1920a) find the potato to compare favorably with roots as a source of vitamin B.

Steenbock and Gross (1919) report the *sweet potato* as con-

taining less of this vitamin than *carrots* or *rutabagas* of which 15 per cent sufficed to make the food mixture adequate. Osborne and Mendel found *turnip, onion, beet leaves*, or *beet stems* to contain about twice as much vitamin (in the dry matter) as *beet roots*, while *tomato* was about twice as rich in this respect as the *turnip* or *onion*, and about half as rich as yeast when all were compared on the basis of dry matter. They also found that 10 per cent of dried *spinach* or 15 per cent of dried *cabbage* sufficed as sole source of vitamin B. Osborne and Mendel (unpublished data) find vitamin B in *lettuce, parsley* and *dandelion leaves*, but not in *tea decoction* as ordinarily drunk.

In general the leaves appear to be slightly richer than the whole seeds in the B vitamin when compared on the basis of dry matter.

Compared in the fresh fluid form, *orange, lemon* and *grape fruit* juice were found by Osborne and Mendel to contain about the same concentration of vitamin B as does milk, while grape juice and fresh apples and pears appeared to contain less.

Nuts. Cajori (1920), working with rats which consumed about 6 to 8 grams of total food per day, found that if almond, English walnut, black walnut, chestnut, Brazil nut or pecan were included in the ration to the extent of 1 to 2 grams per day, the nut sufficed as sole source of the B vitamin. This would imply that nuts are nearly but not quite as rich in vitamin B as are the whole cereals, the dry legumes, or the solids of milk or eggs.

Summary of Properties of Vitamin B.

The term vitamin B at present includes : (1) the antineuritic vitamin found by Eijkman in rice, Grijns in beans, Funk and others in yeast, and later found in a wide variety of animal and vegetable substances, the absence of which causes polyneuritis in fowls, beriberi in man and a similar pathological condition in other mammals; (2) a water-soluble growth-promoting substance found by Hopkins, Osborne and Mendel, and McCollum in milk and later found in wheat embryo, yeast and the various materials furnishing the antineuritic vitamin. Absence of this vitamin causes loss of appetite, cessation of growth and finally pathological symptoms resembling those of beriberi. Similarity in occurrence and many properties speak for the identity of the

antineuritic and growth-promoting water-soluble vitamins. In the absence of positive proof it seems probable that the water-soluble growth-promoting substance is among the substances having antineuritic action.

Vitamin B may also include the *bios* which stimulates the growth of yeast, the auximones or growth-promoting substances apparently synthesized by soil bacteria and necessary for the growth of green plants, and a substance found in blood and other materials and which is necessary for the growth of microorganisms particularly of the hemophilic type.

Pending chemical identification of vitamin B and quantitative experiments in which the same substance is tested for all the above-mentioned properties it is impossible to do more than classify these substances under the general heading of Vitamin B.

Birds fed on polished rice or the corresponding products of other grains develop polyneuritis as the result of the lack of vitamin B. Usually they show considerable loss of body weight at the same time but the nerve symptoms predominate in the pathological picture presented by birds on such a diet.

In mammals a deficiency of vitamin B in the diet causes loss of appetite commonly followed by a more complex and less clear-cut set of symptoms than in the case of birds. These symptoms are grouped by McCarrison in the order of undernutrition, derangement of function of the organs of digestion and assimilation, disordered endocrine function, and malnutrition of the nervous system. A partial but not complete deficiency in this vitamin leads to impaired growth and a general undermining of health and vigor. This lowered vitality may have a far-reaching effect in its influence on reproduction and successful rearing of the young.

While often referred to as growth-promoting, it should be emphasized that vitamin B is essential to normal nutrition at all ages.

The mechanism of the action of vitamin B has been variously thought to be that of a physiological stimulant to glandular secretion; of an indirect metabolic stimulant particularly for carbohydrate metabolism; and of a specific cell nutrient, the nucleopast of McCarrison and Findlay. This latter view is of especial interest in correlating the growth-promoting vitamin essential to normal nuclear metabolism in the body with that

thought by Mockeridge to be essential to the nuclear metabolism in the plant.

While the relation of vitamin B to cellular metabolism seems still a matter of speculation, it is clear that it often exerts a very direct influence upon appetite, as frequently demonstrated by Osborne and Mendel. It is also plain that the vitamin can cure nutritional polyneuritis both in birds and mammals (Funk, Williams, McCollum, Cowgill and Mendel) and both by administration through the mouth and by injection. Thus it has pronounced effect upon the appetite but need not act through the digestive tract.

Hardly less obscure than the mechanism of its physiological action is the problem of the chemical nature of vitamin B. That the substance exists as a chemical entity can no longer be doubted, but as yet it is known only by its physiological effects. Some of its physical and chemical properties have however been determined.

While the discussion of the possible identity of the antineuritic vitamin and water-soluble B has emphasized possible differences in physical and chemical properties, there are certain facts which seem to be established. Vitamin B, the name including both antineuritic and growth-promoting vitamin, is readily soluble in water and in alcohol moderately diluted with water. It is less readily soluble in alcohol stronger than 70 per cent, but can be extracted by stronger alcohol when used hot. It is generally considered to be insoluble in ether, although exceptions to this have been made by Cooper in regard to the antineuritic vitamin in egg yolk, and more recently by Voegtlin and Myers who claim solubility in fat and ether for the antineuritic vitamin of yeast.

Vitamin B is readily adsorbed from solution by fuller's earth, colloidal ferric hydroxide, animal charcoal, and some wood charcoals and probably can be separated from vitamin C by such adsorption. On the other hand both vitamin B and C are dialyzable through membranes of apparently the same permeability (Zilva and Miura, 1921) and, therefore, can not be separated by dialysis. Since both vitamins diffuse through membranes of such permeability as permit the passage of substances of the colloidal character of methylene blue, neutral red, and safranin, Zilva and Miura conclude that the dimensions of the

molecules of vitamins B and C or the molecules with which they may possibly be associated are of the order of that of a semicolloid.

Here as well as in connection with the somewhat conflicting reports regarding solubility in organic solvents it is important to remember that the same vitamin (as identified by its physiological properties) may sometimes be met in a free state as a relatively simple substance and at other times in chemical combination with colloidal substances of presumably more complex constitution. Thus Osborne, Wakeman and Ferry have found evidence that some proteins may combine chemically with vitamin B; and Williams has shown that the antineuritic vitamin of a water extract of rice polishings becomes more active but also less stable when submitted to acid hydrolysis, which would suggest that such hydrolysis sets free the vitamin from chemical combination with some other substance such as protein.

Vitamin B is relatively stable to oxidation and to heat and is evidently more stable in acid than in alkaline solution. Only a few quantitative experiments upon its rate of destruction at different temperatures and under different conditions appear to have been attempted. The results of such experiments (Chick and Hume, 1917b) would suggest that little heat destruction would occur in ordinary cooking, but that in commercial canning and sterilizing the losses might be more serious. In ordinary cooking losses of vitamin B are more likely to occur on account of its solubility in water and consequent loss by extraction if the cooking water is rejected than through instability to heat at cooking temperatures. The distinguishing feature of the B vitamin when compared with vitamins A and C is the effect of its greater stability which tends to make the problem of its ultimate isolation and identification a more hopeful one than that of vitamin C with its marked susceptibility to heat, to alkalinity and to oxidation, and of vitamin A with its susceptibility to destruction by oxidation and the technical difficulties involved in its colloidal and lipoid-like character.

Partly from its solubilities and its greater stability in acid than in alkaline solution and partly from the results of attempts at isolation, as described at some length in the earlier part of the chapter, it appeared probable at a relatively early stage in the study of this vitamin that it was a nitrogenous base very

probably related to the purines or pyrimidines. It is perhaps a hopeful sign that later evidence, both from attempts at its isolation and from studies of its physiological behavior does not in the main contradict the earlier indications. In our opinion further attempts at chemical isolation should be accompanied by quantitative feeding experiments of all fractions obtained, both for the prevention of polyneuritis in pigeons and for the promotion of growth in rats or other mammals. It is chiefly from the substitution of quantitative measurements for the qualitative tests which have been accepted in the past that we may hope for more conclusive evidence in the future.

Williams' attempts to elucidate the problem of the chemical nature of the vitamin by testing substances of known structure for antineuritic properties instead of by depending solely upon fractionation of plant or animal materials, and the resulting indications that antineuritic vitamin may perhaps be a desmotropic form of one of the purines or pyrimidines, are highly suggestive and are not inconsistent with the results thus far reported by Osborne and Wakeman in their work upon the concentration of the vitamin fraction of yeast. We await with much interest the appearance of further work upon this problem, both by Osborne and by Williams.

Chapter III.

The Antiscorbutic Vitamin—Vitamin C.

Theobald Smith in 1895 noted that guinea-pigs kept upon a diet of oats developed a hemorrhagic disease, but the importance of this fact was not realized until several years later.

At about the time that Eijkman and others were showing that beriberi could be induced in fowls as the result of a diet lacking in some definite though unidentified substance, Holst and Frölich (1907, 1912) of Christiania undertook similar experiments with different laboratory animals in the hope of obtaining light upon the disease known as ship-beriberi which was seriously prevalent among Norwegian sailors when on long voyages or fishing trips. Feeling that their experiments would be more directly applicable to human experience if performed upon mammals rather than pigeons or fowls, they gave particular attention to feeding tests upon guinea-pigs. These were found to differ greatly from fowls in their reaction to a polished rice diet. Instead of showing experimental beriberi, the guinea-pigs developed symptoms which Holst and Frölich considered to be "identical in all essentials with those of human scurvy." A diet of other grains or of bread alone resulted in the same symptoms. Among these symptoms are loss of weight, soreness of joints, hemorrhages around rib junctions and knee joints, soreness and hyperemia of the gums leading to looseness of teeth, separation of an epiphysis from long tubular or medulated bones (especially the upper epiphysis of the tibia), characteristic changes of bone marrow, and sometimes hemorrhages into the skin.

To determine whether these symptoms were due to starvation, groups of guinea-pigs were given water alone, water with a limited amount, 40 to 60 grams, of fresh raw cabbage, dandelion leaves, or carrots, and an unlimited amount of the same green vegetables respectively. All of the animals of the first two groups died of starvation but none developed scurvy, while the group fed the fresh vegetables *ad libitum* gained in weight and

showed no symptoms of scurvy. Various foods were then tested for their antiscorbutic properties by adding them to a basal diet of bread or grain, and observing whether or not the guinea-pigs on this diet developed scurvy.

Among the foods showing antiscorbutic properties in varying degrees were raw cabbage, dandelion, lettuce, endive, sorrel, potatoes, carrots, cloudberries, bananas, and apples.

Cabbage and dandelion juices apparently lost their antiscorbutic properties rapidly when kept at room temperature or even in the ice box, while fruit juices and sorrel juice proved more stable. That the stability of the active constituent was greater in an acid than a neutral or alkaline medium was shown by these findings and also by the observation that acidulated cabbage or dandelion juice retained its antiscorbutic property better than the corresponding natural juice.

A study of the effect of cooking on the antiscorbutic property of these materials showed that loss in activity varied with the food and the time of heating. In general, heating for one-half hour at 100° C. proved much less destructive than for one hour at the same temperature.

Cooking cabbage at 110° for one-half hour did not entirely destroy its antiscorbutic properties, but after cooking for one-half hour at 100° and then for one hour at 120° its antiscorbutic properties were almost negligible. Berries retained their efficiency after cooking to a very marked extent. Raspberry juice seemed to be but little injured by heating for one hour at 100° or even 110° C. Cloudberries heated in the same way retained their antiscorbutic properties for at least three months after cooking.

An interesting observation in this paper was that the influence of cooking was apparently less when the air was excluded during the heating. (Compare recent work on destruction of antiscorbutic vitamin by oxidation.)

In most cases vegetables lost the greater part of their antiscorbutic value in drying; but it was observed that such of the antiscorbutic property of cabbage as was not lost in the process of drying was subsequently better preserved in the dry state than when the vegetable was simply stored under ordinary room conditions.

Holst and Frölich in this same article (1912) describe the

production of scurvy in swine by feeding either (1) rye bread, (2) rye bread and cooked beef, or (3) rice and dried cooked fish. In most of these animals a marked polyneuritis developed in addition to the scurvy. It was this same combination of the symptoms of beriberi and scurvy which characterizes the "ship-beriberi," which disease had led Holst and Frölich to undertake these experiments. Thus it appeared that ship-beriberi might be regarded as scurvy complicated with beriberi, and that scurvy might now be studied experimentally in the guinea-pig as beriberi is in fowls and pigeons.

Holst and Frölich showed that scurvy developed in about the same manner when any of the ordinary grains or its mill product is fed. Fürst (1912) working in the same laboratory showed that peas, lentils or almonds, although differing markedly from the cereal grains in percentage composition as shown by ordinary analysis were like the cereals and similar among themselves in their lack of the antiscorbutic substance when fed after ordinary cooking. When peas or lentils were fed raw, scurvy appeared less quickly and in a less severe form, indicating that these seeds contain some of the antiscorbutic substance, but that this is destroyed by heating to the extent required for the thorough cooking of these dry legumes. Fürst also experimented with mixtures of seeds and concluded that "there is no advantage in numerous foods when none contains the needed substance"—an observation which might well become an aphorism for the guidance of menu makers.

Fürst found further that these seeds develop antiscorbutic properties when soaked and allowed to sprout. This is interesting in contrast with an observation recorded by Grijns that seeds lose their antineuritic property on sprouting. These findings have led to the suggestion that the antiscorbutic vitamin may be formed from the antineuritic in the sprouting stage, and that conversely there may be a change of antiscorbutic into antineuritic vitamin as the seed matures and goes into a resting stage.

From this paper by Fürst, as well as from those of Holst and Frölich, one gets the impression that these authors believed in more than one antiscorbutic substance, since the antiscorbutic property seems to differ in stability in different foods. From our present point of view it seems more natural to assume that these

differences in behavior may be due to differing conditions such as hydrogen ion concentration and to colloids whose presence or absence would result in the vitamin being contained sometimes in a heterogeneous and sometimes in a homogeneous system. Nevertheless we know of no evidence adequate to constitute a conclusive demonstration that there is only one antiscorbutic vitamin.

Frölich (1912) held that (under the conditions existing in Norway) infantile scurvy was most common among children living under good hygienic conditions, and that this was probably due to their being fed too largely upon prepared foods and well-sterilized milk, whereas children less sedulously cared for were likely to eat a greater variety of foods, some of them raw, and thus were less liable to scurvy. Since on the other hand the epidemics of scurvy among adults had so often developed under conditions of hardship and unsanitary living, there had been a misleading appearance of these diseases being different whereas in reality they may be essentially identical.

Frölich showed that milk normally contains enough of the antiscorbutic substance to afford complete protection from scurvy when raw milk constitutes the sole or chief food. Such heat treatments as are ordinarily involved in pasteurization of milk, were found in his experiments to diminish but not entirely to destroy the antiscorbutic property so that whether pasteurized milk will furnish enough of the antiscorbutic substance to make the diet safe will depend both upon the treatment to which the milk has been subjected and upon the amount of milk consumed. Pasteurized milk may prevent scurvy in the same manner that raw milk does, provided the pasteurization has been properly carried out and the pasteurized milk is used in sufficiently liberal amounts.

Holst and Frölich (1913) found that cabbage dried quickly in an oven at 37° C. lost little of its antiscorbutic property, and that on keeping it in a desiccator the property was better preserved than when the material was stored in a closed, moist receptacle for the same length of time, thus indicating that destruction of the antiscorbutic substance occurred more readily in the presence of moisture than in its absence. Attempts to extract the antiscorbutic constituent from cabbage by means of

neutral ether were unsuccessful, but it was found to be soluble in alcohol acidulated with 0.5 per cent of citric acid.

A further study of the effect of dehydration upon the antiscorbutic properties of cabbage was reported by Holst and Frölich in 1916 but escaped general notice until recently reported in English. (Holst and Frölich, 1920.)

Slices of cabbage dried for one week in an oven at 37° C., further dehydrated at the same temperature by the action of phosphoric anhydride, and then placed in vacuum bottles and stored at the same temperature showed very pronounced antiscorbutic properties after from 18 to 26 months. Cabbage dried at 37°, but not further dehydrated, lost most of its antiscorbutic properties when kept for 18 months at 37° in closed vessels but retained its properties to a certain extent when kept at from 4° to 12°. Samples kept at 4° in closed vessels without being previously dried showed moderate antiscorbutic properties at the end of 18 months.

Darling (1914) showed in an interesting way by tabulation and diagrams the overlapping of the symptoms of beriberi, scurvy and other deficiency diseases and the extent to which the symptoms of experimental beriberi in fowls and experimental scurvy in guinea-pigs correspond with those of the diseases as observed in man.

The interrelationship of scurvy, beriberi, rickets, and other nutritional diseases, the relative susceptibility of different animals to scurvy, and the striking resemblance between guinea-pig and human scurvy have also been discussed quite fully by Hess in his recent monograph on *Scurvy, Past and Present*. In calling attention to the fact that a diet of polished rice or other decorticated grain will lead to the development of scurvy in the guinea-pig, to polyneuritis in the pigeon or fowl, or to a combination of these disorders in the hog, he suggests that the difference between susceptible and non-susceptible animals is relative rather than absolute. Guinea-pig and human scurvy are considered practically identical, although the guinea-pig is more sensitive to scurvy than man. "This does not indicate that the guinea-pig is an unsuitable experimental animal, any more than the fact that the pigeon is more susceptible to polyneuritis than man indicates that it is unsuited to investigations of beriberi."

In the study of vitamin problems through the evidence ob-

tainable from observations upon disease, two points which are not always clearly distinguished should be kept carefully in mind. From the standpoint of pathology and, to a less extent, of prevention, it is of course of great importance that the experimental disease induced in a laboratory animal shall really be the counterpart of the pathological condition observed in man. At the same time it must be kept in mind that the clinical picture tends to grow with the literature of the disease and that the complete clinical picture may easily include symptoms arising from concurrent infections or other causes aside from the deficiency of the vitamin, whereas in laboratory experimentation it is usually sought to study only one variable factor at a time. Thus in experimental scurvy we may have a picture not showing all of the features which have sometimes been considered as belonging to typical cases of scurvy in man, yet partly for this very reason the experimental form of the disease may be better adapted to the study of the vitamin problem than is its clinical form.

Another important factor in the study of scurvy as a deficiency disease through both experimental and clinical evidence is the possibility that the disease may exist in a latent or sub-acute or partially developed form in which case the patient does not show the complete or typical clinical picture yet may be demonstrably improved by the same measures which are clearly shown to prevent or cure the disease in experimental animals. This is strikingly true in Hess's experience with infantile scurvy. Hess and Fish (1914) described outbreaks of scurvy occurring in an orphan asylum among children who had been fed with milk heated at 165° F. for 20 minutes or milk heated at 145° F. for 30 minutes. No orange juice or other antiscorbutic had been fed. These cases in general did not present a fully developed or clear picture of scurvy but represented only an early stage or a partially developed or sub-acute form of the disease which was, however, responsible for much restlessness, irritability, evident discomfort, and retardation in the growth and development of the children. The condition was cured by the addition of small amounts of orange juice, which, it seems certain, must have been effective because of its antiscorbutic property. Cures were also effected, but less rapidly, by the substitution of raw for pasteurized milk. Modification of the pasteurized

milk by the use of mashed potato made into a thin gruel instead of cereal gruel also served to cure and to prevent the disease. In most of the cases in which the disease developed in children fed pasteurized milk, the milk constituted about two-thirds, and cereal gruel one-third, of the food mixture. On such a diet some of the children developed scurvy at least to the extent here described, while others in the same ward did not, illustrating the fact that susceptibility to scurvy is subject to considerable individual variation or (to state what is probably the same thing from a different angle) that the antiscorbutic requirement for the maintenance of normal nutrition and growth is higher in some children than in others.

In this paper and in another published by Hess in the following year (1915) the pathology of the disease was fully described. Since this and other aspects of scurvy are so well summarized and discussed by Hess himself in his recent monograph (1920), it would be superfluous for us to enter upon any other than the chemical and nutritional aspects of scurvy here.

Ingier (1915) studied the effect of a scorbutic diet upon pregnant guinea-pigs and their unborn young. When the mothers received the scorbutic diets in the early stages of pregnancy, the young were born dead, often prematurely, and on examination showed evidence of impeded growth. When the scorbutic diet was given only during the latter part of pregnancy the young were born alive and apparently fully developed, but with latent scurvy which soon became acute if the mother were continued on the scorbutic diet and the young were dependent upon her milk. It was also observed that pregnant females more quickly succumb to a scorbutic diet than do animals not subject to the demands of pregnancy or lactation.

Commenting upon these results of Ingier, Hess (1920, pp. 126-127) concludes: "In view of the similarity between human and guinea-pig scurvy, we should expect not only miscarriages and still-births to result (when the mother's diet is deficient in antiscorbutic food) but cases of congenital scurvy, especially of the latent or rudimentary type."

An example of the extent to which the growth and development of an infant may be retarded by lack of antiscorbutic vitamin before appearance of distinct scurvy symptoms is afforded by the following case described by Hess (1920, p. 213):

"An infant which had been fed to an age somewhat over nine months without the use of raw milk or other antiscorbutic food was observed to be practically dormant as regards growth, having gained only about one-half pound in four months. It was then fed orange peel juice as antiscorbutic and gained two pounds within a month. There were no other symptoms of scurvy, but the failure of growth followed by prompt resumption when antiscorbutic was given were held to show that the baby was suffering from a progressive scurvy, due to lack of antiscorbutic vitamin and curable by its administration, but not recognizable by any of the classic scorbutic signs.

Jackson and Moore (1916) described experimental studies of scurvy developing in guinea-pigs kept under laboratory conditions on a considerable variety of diets some of which contained foods supposed to be more or less efficient as antiscorbutics. Post-mortem examinations showed hemorrhages in the muscles, ends of bones, bone marrow, skin and tooth pulp; swollen tender joints and fragile bones were also common.

The results of a bacteriological study of these cases were described by Jackson and Moody (1916) in a paper published simultaneously with the above. In the post-mortem examinations which had been made, the staining of certain tissues had suggested the presence of bacteria. Cultures from the affected tissues showed diplococci of low virulence. Rabbits proved more susceptible to inoculations with this organism than guinea-pigs. Only one rabbit died but all showed some stiffness and emaciation. Similar bacteria were recovered from the lesions of inoculated animals as long as 40 days later. When introduced into a second group of animals these organisms failed to produce the disease. Cultures of the heart blood did not show the organisms and blood transfused from diseased to normal animals produced no changes. Animals which had been fed adequate amounts of antiscorbutic food were less susceptible to inoculations with these bacteria than poorly fed animals. These results would seem to illustrate the likelihood that some of the symptoms observed in scurvy may be more or less influenced by the effects produced by infectious organisms which may be in some degree characteristic in the sense that they more readily develop upon a host weakened by scurvy. The fact, however, that a much clearer picture of scurvy develops with greater certainty as a result of

purely dietary causes, confirmed as it is by the great amount of work recently done upon experimental scurvy, leaves no doubt that the disease should be regarded as essentially due to dietary deficiency, though perhaps often complicated by infections and possibly by a particular infection to which the animal becomes more susceptible as a result of the deprivation of the antiscorbutic vitamin.

Chick and Hume (1916-17) in a study of the distribution among foodstuffs, especially those suitable for the rationing of armies, of the substances required for the prevention of beriberi and scurvy, emphasized the fact that the maintenance of adult human beings in health requires an adequate intake of antiscorbutic food as Hess had shown to be the case with children. They distinguished clearly, as Holst and Frölich and Hess had previously done, between two classes of vitamins concerned respectively with beriberi and scurvy, each of which they pointed out has its individual rôle in metabolism possessing properties differing from the other. They also emphasized the difference in distribution of the two substances among natural and commercial articles of food, through a comparison of foods as sources of antineuritic vitamin by experiments upon pigeons, and as sources of antiscorbutic vitamin by experiments upon guinea-pigs, scurvy being induced in the latter by rations of cereals either alone or with sterilized milk. They emphasized the occurrence of the antiscorbutic vitamin in active, living, vegetable tissues, and in smaller degree in corresponding animal tissues. All the dried foodstuffs which they examined, including desiccated vegetables, they reported to be deficient in antiscorbutic vitamin. Dried pulses (legumes) and cereals, though rich in antineuritic, proved too poor in antiscorbutic vitamin to give any protection from scurvy, but if moistened and allowed to germinate the antiscorbutic property was found to be regenerated with the beginning of active cell life in the seed, as previously demonstrated in Holst's laboratory. They therefore recommended that such seeds be made a part of the army provisions to be sprouted and added to the ration when other antiscorbutic food was not available in sufficient quantities. They gave full directions for sprouting the seeds in order to develop the antiscorbutic property and emphasized strongly the fact that

the antiscorbutic vitamin is more sensitive to drying and to high temperatures than is the antineuritic.

In another paper published soon afterward, Chick and Hume (1917) discussed further the independent need of both antineuritic and antiscorbutic substances in the diet at all ages, the absence of antiscorbutic in dry cereals and legumes, its development in either of these groups of seeds when germinated, and the varying proportions of antiscorbutic in different fruits and vegetables. This paper includes a tabulation of different articles of food with notations of their relative antiscorbutic value by means of zero marks or one or more plus signs, a device which has been made increasingly familiar by the successive publications of the English workers and especially by the British Committee Report whose table we publish with slight amendments elsewhere. (Chapter V.)

Baumann and Howard (1917) experimented with the production of scurvy in guinea-pigs by a diet of oats and water and compared the mineral metabolism of the animals in which scurvy had been thus induced with that shown in previous periods during which their diets contained ample antiscorbutic in the form of carrots. They concluded, as had Holst and Frölich, that scurvy developed as a result of the absence or insufficient amount of an unidentified substance in the diet, but further that the disease exerted a profound effect upon the mineral metabolism of the guinea-pig.

Howard and Ingvaldsen (1917) studied mineral metabolism in experimental scurvy of the monkey, this being induced in some but not all of the monkeys by a diet of condensed milk and water. They found changes in mineral metabolism accompanying the symptoms of scurvy in the monkey, but not sufficient to explain the pathogenesis of scurvy, nor were the effects of the scurvy upon the mineral metabolism as marked in the case of the monkey as had been found by Baumann and Howard in the case of the guinea-pig.

McCollum and Pitz (1917) advanced the theory that scurvy in the guinea-pig was more probably due to deleterious effects resulting from constipation than to the deficiency of a specific protective substance. The benefit derived from orange juice they sought to attribute to its laxative effect and possibly also to a more specific influence upon the bacterial flora of the in-

testine. This view considerably influenced the discussion of scurvy during the years 1917 to 1919 but has since been entirely abandoned, at least so far as the chief cause and main features of scurvy are concerned.

Chick, Hume and Skelton (1918) in an investigation of the antiscorbutic value of cow's milk with particular reference to its value as an antiscorbutic in infant feeding, tested the effects of measured amounts of fresh milk fed to young guinea-pigs whose other food consisted of oats and wheat bran.

When less than 50 cubic centimeters of milk was taken daily, the animals did not show protection from scurvy. If the daily ration furnished from 50 to 100 cubic centimeters of milk, more or less perfect protection was observed, varying with the amount consumed. If from 100 to 150 cubic centimeters was taken daily, scurvy was entirely prevented and satisfactory growth and development occurred. These results were considered to agree with the vitamin deficiency theory of the etiology of guinea-pig scurvy, and to show that milk is a food containing a moderate amount of this vitamin. Later experiments have shown that under more favorable conditions of feeding, cows give milk much richer in antiscorbutic vitamin than the above results would indicate.

Chick, Hume and Skelton pointed out that the experiments reported here offer a reasonable explanation of the anomalous results of other observers when guinea-pigs were fed on diets consisting of grain and milk, and no measurements were made of the amount of milk actually consumed. The conclusions of McCollum and Pitz were challenged on this ground, and experiments reported to refute their constipation hypothesis as to the cause of scurvy.

Cohen and Mendel (1918) published in the same year the results of a very thorough and systematic investigation of the relation of diet to the production of scurvy in the guinea-pig. They determined the results of feeding diets restricted to a single seed such as oats, barley or soy bean, and diets in which one or more seeds were fed together with supplements designed to furnish adequate amounts of the different factors known to be needed for normal nutrition. The conditions resulting in the experimental animals were fully described both as to symptoms and autopsy findings. The symptoms regarded by these authors

as most characteristic are tenderness of joints developing usually in the wrists, ankles, and knees in this order, followed in two or three days by swelling of joints or sometimes spontaneous fracture. Fractures were more common among the younger animals. In older ones stiffening or paralysis of the hind legs may occur. The so-called scurvy positions are more or less characteristic. Swellings of the joints may disappear when antiscorbutic is given or they may harden. Hemorrhage of the gums in the experience of these investigators was relatively rare, but loosening of the teeth more common. The condition of the cæcum emphasized by McCollum and Pitz was here found to be not characteristic of the disease, but to depend largely upon other factors in the diet than this antiscorbutic property. With a well-chosen diet the scorbutic animal may still eat well and gain in weight, at least for a time, though at a more advanced stage of the scurvy loss of appetite and of body weight occurs. Susceptibility was found to depend upon age and diet. Guinea-pigs weighing from 110 to 250 grams succumb rapidly and the more so the poorer the diet as regards the character of its protein and mineral content. Animals weighing 300 to 500 grams were considered to be better for the study of most phases of the scurvy problem since younger ones died too quickly. When such animals were kept on a diet of oats and milk, the latter the sole antiscorbutic, it was found that 50 cubic centimeters of milk per capita per day delayed the onset of scurvy for about 75 days, while 85-135 cubic centimeters resulted in complete protection. Contrary to the theory of McCollum and Pitz, Cohen and Mendel found that the roughage of the diet and the resulting intestinal condition were only minor factors, the essential factor certainly being the antiscorbutic substance. Orange juice, fresh cabbage, fresh carrots, adequate amounts of cabbage which had been carefully dried at a temperature of 70°-80° C., germinated oats and barley were all shown to afford protection from scurvy. Pure lactose, which Pitz had believed to be effective through its influence upon the intestinal flora, was here found to be of no value as an antiscorbutic.

Thus it was very clearly shown by Cohen and Mendel that experimental scurvy of guinea-pigs was demonstrable at will with a suitable diet, that it is induced by an exclusive diet of cereal grains as ordinarily fed, but delayed or prevented when

the grains are germinated before feeding. It was rapidly produced by a diet of soy beans supplemented with additions to ensure adequate amounts of both the A and B vitamins together with a suitable mineral content and adequate roughage in the form of added cellulose. In general the findings of Holst and Frölich regarding the antiscorbutic properties of milk and of typical fruits and vegetables were confirmed and it was shown that cabbage could be so dried as still to retain a significant amount of the antiscorbutic substance.

In a paper published simultaneously with that of Cohen and Mendel, Hess and Unger (1918a) furnished further evidence that the constipation theory of scurvy was untenable and that in the guinea-pig scurvy develops upon diets containing both "fat-soluble A" and "water-soluble B" in sufficient amounts to guard against deficiency of either of these vitamins.

In another paper published at about the same time, Hess (1918) showed that in infants no less than in guinea-pigs scurvy may be, and often is, quite independent of constipation. Thus potato though not laxative is a good antiscorbutic while malt soup, although laxative, is a food whose use has been found to be especially prone to lead to scurvy when too great dependence is placed upon it in infant feeding. Hess also offered at this time a very striking piece of evidence against the theory that scurvy is referable primarily to intestinal conditions by showing that scurvy can be cured by intravenous injection of properly prepared orange juice.

Other evidence tending to refute the constipation hypothesis of McCollum and Pitz and to establish the vitamin hypothesis was advanced specifically by Harden and Zilva (1918b), Barnes and Hume (1919), Hart, Steenbock and Ellis (1920) and incidentally by all who during this period were making a special study of the antiscorbutic value of different foods under varying conditions.

With human scurvy admittedly a deficiency disease in that it is caused by the lack of a specific vitamin, it is to be noted as emphasized above that, as in the case of vitamin B, the vitamin may be taken in the food in sufficient amount to prevent the development of the typical symptoms of the deficiency disease, yet not to supply the full need of the body for the vitamin. In such cases there may develop, especially in the growing child,

the condition described by Hess as latent or subacute scurvy, and there may also result a more or less serious injury to the developing teeth as has been emphasized by Zilva and Wells in England and by Howe in this country.

Zilva and Wells (1919) compared microscopic sections of teeth from normal guinea-pigs and from guinea-pigs which had received subnormal amounts of antiscorbutic in their food, and found that the tooth is one of the first, if not the first, part of the system to be affected by a deficiency of antiscorbutic material, and that changes of a profound nature may occur in the teeth even when the ordinary scorbutic symptoms are still so slight as to be almost unrecognizable. The effect upon the teeth was in the nature of a fibroid degeneration of the pulp, a pulpar fibrosis, the fine cellular connective tissue of the normal tooth being replaced by a fibrous structure devoid of distinct cells. Similar radical changes in the teeth have also been noted in monkeys on a scorbutic diet. In discussing the application of these results to human nutrition, the authors suggest the possibility that dietary deficiency is an important factor in the great prevalence of tooth decay in civilized communities. They point out that cases of scurvy so mild as to escape detection may occur more often than is usually suspected and may reasonably be expected to influence dentition.

Howe (1920) also finds that scorbutic diets have a very marked effect upon the teeth, this effect in general resembling pyorrhea. Continuation of the scorbutic diet results in loosening of the teeth; and if this is not allowed to go too far, recovery follows the use of antiscorbutic food.

Hess and Unger (1920a) from clinical observations and post-mortem examination and from the literature on scurvy and rickets conclude that beading of the ribs, the so-called rachitic rosary, is not the result of rickets alone but is one of the typical signs of scurvy and has also been noted in connection with beri-beri and pellagra in children. The belief that this phenomenon is attributable solely to rickets is thought to be responsible for the misinterpretation of many cases of latent scurvy.

McCarrison (1919) found (as did also Rondoni) that in guinea-pigs rendered scorbutic by a diet of oats and autoclaved milk the adrenal glands are enlarged and congested, averaging in the cases reported about twice the weight of normal glands.

He found that this hypertrophy of the adrenals was associated with diminished production of adrenalin which might readily be a factor in the hemorrhages so characteristic of scurvy. La Mer and Campbell (1920) have also found enlargement of the adrenals in scorbutic guinea-pigs.

The universal acceptance of the vitamin hypothesis as related to scurvy was doubtless retarded by the fact that rats which require both vitamin A and B for successful nutrition do not show symptoms of scurvy when subsisting on diets which in human subjects, monkeys and guinea-pigs cause this disease. Harden and Zilva (1918c), however, from studies of the growth and health of rats kept upon diets lacking each in turn of the three vitamins, concluded that "rats existing on a scorbutic diet, although capable of gaining in weight and reproducing themselves, without any apparent manifestation of pathological symptoms for months, do not thrive so well as animals which have their diets supplemented with an antiscorbutic. This suggests that although rats are not very susceptible to scurvy they cannot absolutely dispense with antiscorbutics without restriction of their normal development."

These conclusions were confirmed by Drummond (1919) in feeding experiments with rats through two generations. A larger number of litters of young was obtained from the females which received the added antiscorbutic, which was given in the form of orange juice. (This finding has been confirmed in most but not all of several cases recently tested at Columbia University, the addition of orange juice appearing to exert a favorable effect upon reproduction, even when given to rats whose diet already contained a measurable amount of antiscorbutic in the form of milk powder.) Drummond concluded from his observations that "the rat requires the antiscorbutic factor in order to achieve a normal development, and that although the requirements of this species are of a very much smaller order than those exhibited by man, the monkey, or the guinea-pig, they are sufficiently well marked to dispel any idea that there exists a fundamental difference in the nutritive requirement of the two types of animals.

"It may therefore be accepted as experimentally proven that the dietary requirements of the higher animals include in addition to a satisfactorily balanced ration of protein, fat, carbohydrate, and mineral salts, an adequate supply of three accessory

food factors: Fat-soluble A, water-soluble B or antineuritic factor, and water-soluble C or antiscorbutic factor."

The susceptibility of the monkey to scurvy and its requirement for vitamin C as compared with the requirements of the guinea-pig and of man have been studied by Harden and Zilva (1919, 1920). In the first of two papers on this subject (1919) three experiments were reported in which scurvy was induced in the monkey by the following diets: First, fresh beer, steamed wheat germ, and autoclaved bread, rice and peanuts; second, autoclaved rice bread and autoclaved milk; and third, a diet similar to the first with the substitution of autoclaved milk for the beer. In all cases an acute scorbutic condition developed in from three to four months, the symptoms in the first two cases being confirmed by the histological changes noted on autopsy. In the third experiment the animal was cured in less than a week by the feeding of lemon juice. The minimum protective dose of orange juice for monkeys was later (1920) found to be between 1 and 2 cubic centimeters per day while doses of 2 to 5 cubic centimeters proved an adequate prophylactic.

"A monkey therefore of 2 or 3 kilos is protected from scurvy by about the same quantity of orange juice as a guinea-pig weighing 300 to 400 grams. It is interesting to note that while the minimum dose of antiscorbutic required by the two animals to protect them from scurvy is of the same order, the time taken for the development of the disease is very different, being about two months for a monkey and three weeks for a guinea-pig. This suggests that the monkey possesses a higher store of the antiscorbutic factor than the guinea-pig, while their daily requirements for metabolism are equal."

It is estimated that an average child of 8 to 10 kilos needs as much antiscorbutic vitamin as is furnished by about 15 cubic centimeters of orange juice per day. This indicates about the same requirement per unit of body weight in the child as in the monkey, while the guinea-pig's requirement per unit of weight is about five times larger. This is probably only a very rough approximation.

While it is generally conceded that the antiscorbutic vitamin can not be stored in the body to the extent that is possible with vitamin A (Hess, 1920, p. 275) the suggestion of Harden and Zilva as to the relative storage of the antiscorbutic vitamin in the

monkey and the guinea-pig is of interest as offering a possible explanation of the varying requirements of different species of animal.

Of great importance in this connection is the recent demonstration by Parsons (1920) of the persistence of the antiscorbutic substance in the liver of the rat after long intervals on a scorbutic diet. In an effort to explain the apparently marked differences in antiscorbutic requirements of the two rodents, the guinea-pig and the rat, the antiscorbutic content of the rat's body tissues, (1) when a typical scurvy diet was fed and (2) when the diet was high in antiscorbutic substance, was tested by feeding extracts of the rat liver and muscle tissues to scorbutic guinea-pigs. For the purpose of comparison fresh fish tissue was also used.

The liver tissues of the rat showed a high content of the antiscorbutic substance, not only after feeding for a short time on a diet rich in the antiscorbutic substance but also after feeding for a long time on a typical scorbutic diet, practically no difference in the growth curves of the guinea-pigs on the two diets being noticeable. The muscle tissues of the rat could not with one exception be fed at an intake high enough to effect a cure in the scorbutic guinea-pig. In one case when the intake of the muscle extract reached the equivalent of from 55 to 95 grams per day the scurvy symptoms were somewhat improved. The water extract of fresh fish muscle tissues equivalent to 35 grams of the tissue proved incapable of curing scurvy.

These results are thought to indicate the need for the antiscorbutic vitamin in the normal metabolism of the rat, thus confirming the conclusions of Harden and Zilva and of Drummond, and to suggest the possibility that the rat is capable of synthesizing this substance. Other possibilities suggested and discussed are the utilization by the rat of undemonstrable amounts of the antiscorbutic substance in the food or of a form not available to the guinea-pig.

Occurrence and Properties of Vitamin C in Foods of Vegetable Origin.

Impetus to a systematic study of the occurrence of the antiscorbutic vitamin in food materials and its stability under different treatment was given by the tremendous problem of civilian

and army rationing during the World War. In spite of a better understanding of preventive and curative measures, scurvy played an important rôle in the nutrition of the troops, particularly on the Eastern front, and also in the civilian population of central and eastern Europe during and after the war.

A striking illustration of the relationship of beriberi and scurvy to particular dietary deficiencies is afforded by the reports of Willcox (1917, 1920) and of Hehir (1919, 1919a), of the outbreaks of beriberi in the British troops and scurvy in the Indian troops in Mesopotamia in 1915 and 1916, particularly during the siege of Kut-el-Amara. Hehir reports that during this siege there were about 1050 cases of scurvy, all but one of which were in the Indian troops, while beriberi appeared among the British, and not among the Indian troops.

The high incidence of scurvy among the Indian troops and almost complete absence of it among the British troops is traced to the use by the latter of fresh meat toward the end of the siege when the bullocks, horses, and mules were killed to eke out the diminishing food supplies. The Indians who did not overcome their scruples against eating horseflesh were the worst sufferers from scurvy. In the last period of the siege, although the men were rapidly losing weight on a starvation diet, the disease declined coincident with the use of about 3 ounces per man per day of green herbs collected from the plains. It is emphasized that fresh meat alone without vegetables will not indefinitely postpone scurvy but seems to delay its appearance. An increase in the meat rations for the Indians and the development of vegetable gardens for the stationary troops caused scurvy practically to disappear from Mesopotamia. Willcox in commenting on this same outbreak attributes the greater immunity to scurvy of the white troops in part to the slight antiscorbutic value of the fresh meat which they consumed and in part to the fact that they had previously been accustomed to a varied diet containing ample antiscorbutics whereas the previous food habits of the Indian troops had resulted in their being in many cases already on the border-line of scurvy. While the fresh meat was believed to have undoubted value it was of course here, as in much other experience, recognized as being but a poor antiscorbutic.

The occurrence of beriberi among the white troops and com-

plete absence of it in the Indian troops during the same siege is attributed chiefly to the use of ration biscuits or white bread by the former and atta (flour containing the wheat embryo) by the latter troops. One of the measures taken to improve the dietary of the British troops was the incorporation of 25 per cent of atta in the bread.

The urgent need of more exact knowledge of the antiscorbutic values of foodstuffs of practical war-time utility led to a systematic survey (principally at the Lister Institute, London, and simultaneously to a lesser extent in other laboratories) of the commoner foodstuffs with respect to their relative value as antiscorbutics, and to studies of methods of stabilizing and concentrating the vitamin for practical use.

The methods used at the Lister Institute for testing antiscorbutic values were essentially a development of those of Holst with the exception that a daily ration of 60 cubic centimeters of milk autoclaved at 120° C. for one hour was added to the basal ration of oats and bran. This addition was found to improve greatly the general condition of the guinea-pigs without greatly influencing the onset of scurvy. In view of later findings it appears (Hess, 1920) that small but varying amounts of the antiscorbutic vitamin may remain in such autoclaved milk which would detract somewhat from the exact quantitative value of the results.

Fruits and Fruit Juices.

A study by these methods of the relative antiscorbutic values of the juices of *limes* and *lemons* (Chick, Hume and Skelton, 1918a) led to the conclusion that lemon juice is about four times as rich in antiscorbutic vitamin as is lime juice, and that preserved lime juice is not to be depended upon for prevention of scurvy. These results are of interest in view of the traditional use of "lime juice" as an antiscorbutic. Historical investigation (A. Henderson Smith, 1918) indicated that the actual material to which the term lime juice was applied was frequently the juice of lemons and not of limes and that it was really to lemon juice that the practical elimination of scurvy from the British navy and merchant marine had been due. As a striking instance of this the case is cited of two British expeditions into the Arctic, that of the *Investigator* in 1850 which reported wonderful immunity to scurvy throughout two years of privation during which,

however, lemon juice was available as antiscorbutic and that of the *Alert* and the *Discovery* in 1875 which used lime juice and had trouble with scurvy before the end of the first winter.

Harden and Zilva (1918a) selecting *lemon juice* as a convenient material for studying the concentration of the antiscorbutic vitamin, found that the liquid remaining when citric acid had been precipitated from the juice by the addition of calcium carbonate appeared to contain most if not all of the vitamin. The method used was as follows: To the fresh lemon juice was added with constant stirring an excess of precipitated calcium carbonate followed by two volumes of absolute alcohol. The mixture was then filtered, the residue pressed in a hand press and the liquid thus obtained filtered; the filtrates were combined, evaporated in vacuo at 35° C. to remove all alcohol, and made up to the original volume with distilled water. The solution thus obtained, which is sometimes referred to as "treated juice," was yellow, acid to litmus, sweet to the taste and lemon flavored. This liquid showed some deterioration after storage in the cold for about a fortnight and marked deterioration on evaporation at 30° to 40° in vacuo. If, however, the treated juice was slightly acidified by the addition of citric acid and then evaporated in vacuo at 30° to 40° as before, a residue of high antiscorbutic potency was obtained.

By the use of this preparation it was possible to feed larger doses of antiscorbutic than in the form of ordinary foods. The administration of liberal doses of treated lemon juice previous to depriving guinea-pigs of the antiscorbutic factor did not seem to prevent nor delay the onset of scurvy, thus indicating that the antiscorbutic vitamin is not stored in the body. Since, however, the observations of Parsons (1920) have led her to suggest that storage in the body may be an important reason for the apparent differences in requirement in different species, it would be premature to generalize broadly on this point. The treated juice not only proved potent as an antiscorbutic for guinea-pigs and monkeys (Harden and Zilva, 1918a, 1919) but brought about prompt recovery from scurvy in children seven months to one year old (Harden, Zilva and Still, 1919). The rapidity of recovery was attributed to the fact that it was found possible to give the antiscorbutic in amounts equivalent to the juice of from 6 to 12 lemons daily without any gastro-intestinal dis-

turbances, even in the case of children less than a year old. These authors consider that, if any evidence were still needed of the essential identity of human scurvy and the experimental scurvy of the guinea-pig, the parallelism of results obtained with this preparation representing only a fraction of the juice of the lemon would now leave little room for doubt as to the bearing upon human scurvy of results obtained in experimental scurvy with monkeys and guinea-pigs.

The "treated" or "decitrated" lemon juice has since been used to a considerable extent as a prophylactic and therapeutic measure and also in experimental work on the antiscorbutic vitamin.

Another concentrated preparation of lemon juice has been recently described by Bassett-Smith (1920). The fresh juice is filtered through muslin and then through filter paper under reduced pressure. The filtered juice is evaporated in vacuo over sulfuric acid at ordinary temperature and the sirupy residue made into as stiff a paste as possible with a mixture of 97 per cent anhydrous lactose and 3 per cent gum tragacanth. The paste is cut into sections, each containing the juice of half a lemon. These are then rolled and pressed into the shape of lozenges. Experiments are reported in which these tablets were used both for the prevention and for the cure of scurvy in guinea-pigs with excellent results, one-fifth of a tablet, equivalent to 4.8 cubic centimeters of fresh lemon juice, representing the minimum preventive dose. It is estimated that one tablet, the equivalent of half a lemon, would be ample as a daily dose to prevent scurvy in adult human beings.

As these tablets have been found to retain their antiscorbutic properties for over a year when stored at room temperature (Bassett-Smith, 1920a, 1921) they afford an antiscorbutic of small bulk eminently suited for long voyages. Macklin and Hussey (1921) state that the antiscorbutic substances used on board ship in the Shackleton polar expeditions include lemon juice concentrated by this method but not made into tablets, while for sledging conditions lemon juice tablets are used.

Harden and Zilva (1918) also made use of *orange juice* in their studies of the behavior of antiscorbutic substances. On treating a mixture of autolyzed yeast and orange juice with various adsorbents and testing the filtrates on polyneuritic pigeons and scorbutic guinea-pigs, they found that while fuller's

earth and dialyzed iron adsorbed almost all of the antineuritic vitamin, such treatment did not affect the antiscorbutic activity. Similarly, orange juice did not lose its activity on filtration through a Berkfeld candle.

Harden and Zilva (1918d) also studied the susceptibility of the antiscorbutic substance to alkalinity by experiments upon guinea-pigs receiving oats, bran and autoclaved milk with measured addenda of orange juice which was fed in some cases fresh and unchanged, in other cases neutralized immediately before feeding, while in still other cases the juice was made alkaline and allowed to stand for some time before being fed. Of freshly expressed orange juice, from 3 to 5 cubic centimeters was always sufficient to keep guinea-pigs in good condition and free from any symptoms of scurvy. Three guinea-pigs receiving respectively 3, 5, and 7 cubic centimeters of orange juice which had just been rendered neutral to phenolphthalein were also protected from scurvy. On the other hand, doses of 3, 5, and 7 cubic centimeters of an orange juice which had been made twentieth-normal alkaline by addition of sodium hydroxide and then allowed to stand 24 hours in a cold room before feeding did not protect. That this was due to destruction of the antiscorbutic vitamin and not to any effect of the alkali upon the animal was shown by the fact that the results were unchanged when the juice which had been thus treated was reacidified immediately before being fed. In a final experiment to test the effect of less drastic treatment, the juice was made fiftieth-normal alkaline by addition of sodium hydroxide and later in the same day fed in doses of 3, 5, and 7 cubic centimeters respectively to three guinea-pigs. All died of scurvy, though not so quickly as those receiving the basal diet only, showing that a considerable part but not all of the antiscorbutic vitamin was destroyed by this treatment.

Hess (1918c) reported that orange juice, boiled and slightly alkalinized, constitutes an excellent antiscorbutic agent for intravenous use in infantile scurvy, but in such cases the juice should be used immediately and not kept in the alkaline condition. Instability of the antiscorbutic vitamin to alkali was demonstrated by Hess and Unger (1919) who found in their clinical experience that modified milk mixtures containing malt soup were especially prone to bring about scurvy in infants. On investigation this was traced to the practice of adding alkali to the malt soup

and thus with it to the food mixture where it exerted a deleterious effect upon the antiscorbutic vitamin of the milk. This was demonstrated (1) by curing a child (whose scurvy had evidently been brought about in this way) by simple omission of the alkali from the formula used in modifying the milk, and (2) by experiments upon guinea-pigs.

Hess and Unger (1918a) in a study of guinea-pig scurvy reported that orange juice lost some of its antiscorbutic power after storage in a refrigerator for three months, that the antiscorbutic substance of orange juice could be extracted with 95 per cent alcohol, and that orange peel after being dried and kept dry for three months still showed antiscorbutic properties. Prunes seemed in their experiments to have no value as an antiscorbutic, and the question naturally suggests itself whether the practice of dipping prunes in alkaline solution in connection with the drying process is the cause of their deficiency in vitamin C.

Harden and Robison (1919a) showed that the antiscorbutic substance in orange juice is not volatilized nor largely destroyed when the juice is distilled at 40° C. under reduced pressure. The solid residue obtained by this process was found to have strong antiscorbutic properties which were not appreciably diminished on keeping the substance in a dry atmosphere at room temperature for six months.

Lime juice concentrated in the Kestner evaporator under reduced pressure was found to retain nearly all of its antiscorbutic value, though the acidity of the concentrated juice rendered it rather unsatisfactory in the guinea-pig experiments. Apple juice concentrated in the same manner to about one-sixth of its original volume formed a soft palatable jelly of distinct antiscorbutic properties, although not so valuable an antiscorbutic as the dried orange juice.

The general use of fruit jellies prepared by this or similar processes is recommended as a substitute for ordinary jams or jellies wherever there is reason to suspect that the diet is deficient in the antiscorbutic vitamin.

Later (Harden and Robison, 1920) samples of dried orange juice prepared as described above were tested for antiscorbutic potency after having been stored in a desiccator at room temperature for nearly two years. Complete protection from scurvy

was afforded guinea-pigs weighing about 300 grams by a daily ration of 0.5 gram of the dried orange juice equivalent to about 4.5 cubic centimeters of the raw juice. Similar samples of dried juice (1921) lost about 50 per cent of their antiscorbutic properties after storage in a dry atmosphere at 29° C. for 15 months.

Givens and McClugage (1919a) dried orange juice in two different ways as follows:

For one product, the juice was expressed from the oranges and strained through several layers of cheese cloth until a clear filtrate was obtained. This was concentrated to about two-thirds the original volume by heating in a drier at from 55° to 60° C., using 100 cubic centimeter portions in small shallow dishes. Soy bean flour, previously heated under 20 pounds pressure for one-half hour, was then added in the proportion of 12 grams to 100 cubic centimeters of the original juice and mixed thoroughly. The mixture was kept in the drier at from 55° to 60° until thoroughly dried, the whole process requiring about 50 hours. In this case the antiscorbutic vitamin was largely but not entirely destroyed.

For the other product, the juice was dehydrated by a commercial process employed for making milk powder. The juice was pressed from the oranges, strained, mixed with corn sirup, and dried by spraying into a chamber kept at from 75° to 80° C. In this process the juice was dried almost instantaneously and the product was actively antiscorbutic. The authors therefore recommend that in drying the juice, the temperature used should not be unduly high and the time of heating should be short. Juice so dried as to retain its antiscorbutic power was still active after three months and has later (Givens and Macy, 1921) been found to be active after from 14 to 20 months.

Further studies of the juices of the orange, lemon and lime have recently been reported by Davey (1921). The minimum daily doses of the three citrus fruits needed to protect the guinea-pig from scurvy were established as lemon and orange 1.5 cubic centimeters, and lime 5 cubic centimeters. These values were used as a basis for comparing the antiscorbutic properties of the juice of oranges and lemons kept for varying times at different temperatures, alone, with the rind oil, and in the case of lemon juice with the addition of sodium sulfite. Preservation with sulfite appeared to be satisfactory at low temperatures, uncer-

tain at room temperature, and unsatisfactory at 37° C. Preservation with the rind oil was satisfactory and reliable at about 0° C. and room temperature, but unsatisfactory at 37°. Experiments on the preservation of oranges and lemons in cold storage were not particularly satisfactory on account of the fact that the fruit did not keep well in these particular cases for any length of time, but the results indicated that the antiscorbutic property was not seriously diminished so long as the fruit remained edible.

Grapes appear to have received surprisingly little attention. Chick and Rhodes (1918) report that scurvy developed in three of four guinea-pigs receiving 20 grams each of grapes daily. This would therefore appear to approximate the minimum protective allowance and to indicate that the concentration of vitamin C in grapes is about one-tenth as great as in oranges. Givens and Macy (1921) state that grape juice dried by the spray process shows no antiscorbutic properties.

The tomato, while ordinarily referred to as a vegetable, is more properly a fruit and may well be considered at this point because of the similarity of tomato juice to orange juice as an antiscorbutic. Hess and Unger (1918) called attention to the importance of tomatoes, whether fresh or canned, as antiscorbutics and showed that canned tomato juice is a very efficient, readily available, and inexpensive antiscorbutic which may be used in the same manner and with practically the same results as orange juice, even in the feeding of infants. Hess (1920) says: "In our experience there is no contra-indication to the giving of orange juice or of strained canned tomato, the two antiscorbutics with which we have had a large experience, to babies one month of age or even younger." And again "Tomatoes . . . are regarded with suspicion amounting almost to superstition by mothers and nurses as a food for children. In spite of this fact, it may be stated without hesitation that they are fully as well borne by infants a few weeks or months of age as orange or lemon juice. . . . The dose is two tablespoonfuls for babies over three months of age. The tomatoes are merely strained through a colander and warmed (not cooked). To illustrate their innocuous character, it may be added that as much as 6 and 8 ounces a day of this juice have been given to a baby under one year of age without producing untoward symptoms. This antiscorbutic

should have a wide applicability, especially in the United States."

It is probable that the antiscorbutic property of the canned tomato has been a factor (even though not consciously recognized) in the popularity of this food among, for example, the city poor as well as among those economically able to command a more diversified diet. A similar significance may be attached to the provision introduced into the United States Army Regulations in 1895 permitting requisition of canned tomatoes "in lieu of an equal quantity of potatoes not exceeding twenty per cent of the total issue." Hess records (1920, p. 231) his inability to ascertain whether or not the canned tomatoes were thus provided for specifically as an antiscorbutic.

Largely no doubt on account of their natural acidity, tomatoes may be canned without apparent loss of antiscorbutic value, and may even retain a relatively high antiscorbutic value after drying.

Givens and McClugage (1919) found that when 1 gram of dried tomato which had not been heated above 40° C. was fed per guinea-pig per day no scurvy developed. Since this may have been considerably more than the minimum necessary for complete protection, these experiments show clearly that tomatoes may retain a considerable amount of antiscorbutic vitamin after drying; but do not indicate what proportion of the vitamin originally present may have been destroyed by the drying process.

In the experience of Hess and Unger (1918), 4 cubic centimeters, and in that of Sherman, La Mer and Campbell (1921), 3 cubic centimeters daily of canned tomato juice was found to afford the guinea-pig complete protection from scurvy. In tomato juice of natural acidity, the antiscorbutic vitamin was found to be fairly stable to heat, boiling for one hour destroying only about one-half; and boiling for four hours destroying only about two-thirds of the vitamin originally present. When the acidity was partially neutralized, and still more when the tomato juice was rendered alkaline, the destruction of the vitamin by heat became more rapid (Sherman, La Mer and Campbell, 1921). The rate of destruction of the antiscorbutic vitamin of tomato at different temperatures and concentrations of hydrogen ion will be referred to again in connection with the summary of physical

and chemical properties of vitamin C toward the end of this chapter.

Berries are much sought as antiscorbutics in Northern regions but appear to have been little studied. Holst and Frölich (1912) report raspberries and cloudberries to be good antiscorbutics and state that their juices can be boiled, canned and kept for months with relatively little loss of antiscorbutic potency.

In so far as comparison can be attempted on the basis of present knowledge it would appear that citrus fruits and acid berries show higher antiscorbutic value than those fruits which are more starchy or richer in sugar, and the latter if dried before being sent into commerce and then handled without refrigeration naturally lose more or less of whatever antiscorbutic potency they originally possessed.

Lewis (1919) investigated the antiscorbutic value of the *banana* by tests upon young guinea-pigs weighing about 300 grams. The amount required for the prevention of scurvy was found to vary with the adequacy of the basal diet. Guinea-pigs on an exclusive diet of banana were protected from scurvy but were unable to maintain body weight and did not live longer than 20 to 30 days. Banana in excess of 25 grams per guinea-pig per day as a supplement to rolled oats was adequate to protect against scurvy but this diet did not give normal growth. A diet of autoclaved oats, bran, milk, casein, and salts was tried in order that the basal diet might be adequate in all respects except for antiscorbutic. With this basal diet from 10 to 15 grams of banana per guinea-pig per day proved adequate both for growth and for the prevention of scurvy. From this the conclusion was drawn that a lower amount of antiscorbutic was required when the basal diet was otherwise adequate. This is doubtless true and is an important fact to bear in mind in the interpretation of experiments upon the antiscorbutic properties of different foods. In this case, however, it seems probable that the milk used in the basal diet, although dried in the preparation of the food mixture in the laboratory, may very likely have furnished some antiscorbutic as fed, in which case the results obtained on this basal diet may have been somewhat unduly favorable to the estimate of the antiscorbutic value of the banana.

A recent report of Givens, McClugage and Van Horne (1921)

states that 10 grams of raw *banana* or *apple* provided sufficient vitamin C for the prevention of scurvy in the guinea-pig.

Chick, Hume and Skelton (1919) were led to investigate the value of some Indian dried fruits through the fact that these foods enjoyed the reputation of antiscorbutics in India and have been relied upon to some extent by native Indian troops. The fruits used in this investigation were *tamarinds, cocum* and *mango.* Specimens were obtained from India and experiments were performed upon guinea-pigs according to the usual methods of these English investigators. A basal diet consisting of oats and bran with 60 cubic centimeters autoclaved milk per animal per day was used. The fruit to be tested was fed in addition to this diet. As the animals would not eat the food in its original form, it was made into a thick syrup and fed by means of a syringe. The quantities thus fed were equivalent to 3 to 5 grams of the dried food per animal per day which quantities showed antiscorbutic effects but did not suffice for complete protection. The conclusion was drawn that dried tamarinds, cocum and mango possess significant but not large quantities of the antiscorbutic vitamin, that weight for weight these fruits are superior to carrot, cooked potato, or raw meat juice, but greatly inferior to raw cabbage, germinated pulses or the juice of Swedish turnips, oranges or lemons.

Roots and Tubers, Bulbs, Stems and Leaves.

Among vegetables, raw greens and some of the roots and tubers enjoy high reputation as antiscorbutics.

The *Swedish turnip,* rutabaga or "swede" has been studied by Chick and Rhodes (1918) who found that the juice obtained by filtering the freshly grated pulp through a muslin bag approximated orange juice in antiscorbutic potency. A daily ration of 2.5 cubic centimeters of this juice fully protected a guinea-pig against scurvy while to afford approximately the same amount of protection required about 20 cubic centimeters of carrot juice and still more of beet juice. Raw "swede" juice has therefore been widely advocated by the English workers as an inexpensive source of vitamin C particularly in infant feeding.

Carrots have been studied by Hess and Unger (1919) who find a distinct difference in the antiscorbutic properties of old as compared with fresh young carrots particularly after cooking.

It was found that while 35 grams of old carrots were sufficient to protect a guinea-pig from scurvy when fed raw, after cooking for three-quarters of an hour their addition to the dietary proved insufficient for protection. In a parallel test with fresh young carrots 25 grams proved adequate for complete protection even after cooking. It is pointed out that in such cases as this the fresh young vegetable may have a double advantage over those which are older and tougher, since in the first place the younger or fresher specimens may be richer in antiscorbutic vitamin to start with and as the older, tougher vegetables require more prolonged cooking they are apt to experience a greater loss of antiscorbutic during the cooking process.

Hess also points out (1920, p. 160) that the loss of antiscorbutic value in the boiling of carrots is due to destruction and not merely to extraction of the antiscorbutic vitamin since the water in which the carrots had been cooked was found by feeding experiments to have little if any antiscorbutic value. This is in contrast to observations showing considerable amounts of vitamin B in the "water" of cooked or canned vegetables. The difference is probably due to the greater susceptibility of the antiscorbutic vitamin to heat, which would result in its destruction rather than its accumulation in the boiling water surrounding the cooking vegetable, whereas the equally soluble and more stable vitamin B would accumulate in the cooking water to a much larger extent. Less easily understood is the further finding by Hess that the acidulation of the cooking water with vinegar did not reduce the loss. Foods naturally acid seem always to retain their antiscorbutic property relatively well under heat treatment, while attempts to conserve the antiscorbutic vitamin of other foods by addition of acid seem to give variable results.

Largely as the result of his experience with carrots, Hess (1920, pp. 160, 161) lays much emphasis upon the view that vegetables must be expected to vary considerably in their content of antiscorbutic vitamin according to their freshness and age. While young carrots were much superior to old carrots in antiscorbutic value, he found that slightly green tomatoes contained less antiscorbutic vitamin than did those which were fully ripe.

So far as fruits and vegetables are concerned, our present

limited knowledge would seem to suggest that the degree of maturity at which the fresh food is most prized will probably approximate that at which it has greatest vitamin value.

Dried carrots were found by Hess and Unger to have lost antiscorbutic vitamin to a serious extent but not necessarily entirely. In the quantities which they fed, the carrots which had been allowed to age before drying failed after drying to prevent scurvy, whereas protection was afforded by an equal amount of carrot which had been quickly dried when young and fresh. In this connection as well as elsewhere, Hess emphasizes the view that the length of time during which the vitamin is exposed to any unfavorable condition such as heating or alkalinity "is in general more important than the intensity of the process"; and that "too much attention has been paid to the degree of the heating process and too little to the more important factors—the age of the vegetables, their freshness previous to dehydration, their manner of preservation."

The emphasis thus laid by Hess upon the importance of the time factor in problems connected with the destruction or conservation of antiscorbutic vitamin will be discussed further in connection with other experiments to be described beyond.

Potatoes while containing the antiscorbutic vitamin in distinctly lower concentration than do oranges, lemons and tomatoes, are yet of great importance as antiscorbutics because of the quantities in which they are consumed. Hess (1920) estimates that in the countries of the temperate zone the consumption of potatoes is probably twice that of all other vegetables combined and that for this reason our protection from scurvy actually rests very largely upon the potato. "Therefore if this crop fails scurvy will develop in the spring." Weight for weight, cooked potatoes are comparable as antiscorbutics with apples and bananas and have only one-fifth to one-tenth of the concentration of vitamin C which we find in oranges, lemons and tomatoes. According to Holst and Frölich (1912) 20 grams per day of potato cooked by steaming for 30 minutes at 100° C. fully protect a guinea-pig from scurvy.

Givens and Cohen (1918) reported a number of experiments with potatoes cooked in different ways and in some cases dried before feeding. From the variable results obtained the authors were led to suggest the possibility that the factors involved in

the destruction of the antiscorbutic vitamin are not only the degree of heat and the duration of the heating but also the enzyme content and the reaction of the food being dried. By employing a high temperature for a short time, as in the case of baked potatoes, the enzymes are destroyed, while at any temperature below 80° C. the enzymes are still functioning and may perhaps play a rôle in the destruction of the antiscorbutic vitamin.

In a later paper Givens and McClugage (1920a) reported further work on the antiscorbutic value of potatoes, raw and cooked, fresh and dried. Young guinea-pigs were fed a basal scurvy-producing diet of heated soy bean flour, milk, yeast, paper pulp, calcium lactate, and salt, and to this was added 10 grams daily of fresh potatoes or its equivalent in the dried or cooked product.

A daily supplement of 10 grams of raw white potato was sufficient to protect the growing guinea-pigs from scurvy for the duration of the experiment, 129 days. While the minimum protective amount of raw potato was not determined, indications are that slightly less than 10 grams daily is about the lower limit of safety. Cooking the potatoes in water at 100° C. for 15 minutes caused only a slight reduction in antiscorbutic value, while cooking for an hour at the same temperature reduced the vitamin content to such an extent that the disease could not be arrested by feeding 15 grams daily of the product. Scurvy was checked in two animals by feeding 10 grams of potatoes cooked in 0.5 per cent citric acid for 1 hour.

With potatoes dried at 35° to 40°, death from scurvy was slightly delayed by an amount (2.5 grams) equivalent to 10 grams of the fresh product, while with double the amount life was prolonged still further. One out of four animals on a daily dose of 2.5 grams dried at 55° to 60°, and one out of four animals on a daily dose of 2.5 grams dried at 75° to 80°, showed signs of scurvy at death. On heating at 100° for 1 hour the products dried at these temperatures, no protection was secured in any case.

Onions are reported good antiscorbutics by the British Committee. See also Shorten and Roy (1919).

Stems such as asparagus and celery would seem to be worthy of attention as regards antiscorbutic value but do not seem to

have been studied. *Rhubarb* was shown to possess antiscorbutic power by Pierson and Dutcher (1920).

Leaves are apparently good antiscorbutics but only a few of them have been studied. Lind gave directions for the use of fir tops as antiscorbutic in time of need, and referred to "scurvy grass," cress and spinach as foods of known antiscorbutic value.

Holst and Frölich (1912) reported *cabbage* and *lettuce* as possessed of good antiscorbutic properties. Of these the cabbage has been much the more extensively studied, doubtless because it is quantitatively the most important of our leaf vegetables and perhaps in part also because it is so commonly fed to, and so readily eaten by, the guinea-pig which usually serves as the experimental animal in studies of the antiscorbutic vitamin.

Reference has already been made to the experiments of Holst and Frölich with raw, cooked and dried cabbage. The work of Delf (1918; also Delf and Skelton, 1918) indicates that with a good basal diet from 1.5 to 2 grams of raw cabbage will suffice to prevent scurvy in a guinea-pig, thus pointing toward as high a concentration of the antiscorbutic vitamin in the raw leaf as in the juice of the orange or lemon and somewhat higher than in the tomato. In practice, however, cabbage is probably a less important antiscorbutic than tomato both because the bulk of fiber in the cabbage tends to limit the amount consumed, and because the cabbage loses a much larger fraction of its vitamin C in cooking than does the tomato.

Delf (1918) reported an extended investigation on the antiscorbutic and growth-promoting value of raw and heated cabbage as determined by experiments upon guinea-pigs. The basal diet used was a mixture of rolled oats and bran *ad libitum* with water and, in cases where the rations of cabbage were small, with a daily supplement of 60 cubic centimeters of milk autoclaved for one hour at 120° C. The cabbage was given in the green leaf. One gram of fresh raw cabbage was regarded as a minimum daily dose for protection of the guinea-pig from scurvy. For entirely satisfactory results, however, larger quantities were generally necessary. In a large number of experiments in which the basal ration included 60 cubic centimeters autoclaved milk and in which from 1.5 to 5 grams of cabbage were added daily to the diet nothing more than incipient scurvy was observed. Better results with normal growth and complete absence of any

suggestion of scurvy were obtained when the allowance of cabbage was increased to 30 grams per day.

Heating experiments showed the antiscorbutic value of cabbage to be relatively sensitive to temperatures of 60° and upward but the temperature coefficient of the heat destruction of the vitamin appeared rather low, an increase of 30° to 40° apparently increasing the rate of destruction only about three fold, as shown by the fact that 5 grams of cabbage cooked for one hour at 60° was about equal in antiscorbutic value to 5 grams cooked at 100° for 20 minutes or to one gram of raw cabbage.

Applying these results to a discussion of methods of cooking vegetables the author pointed out that slow cooking at a low temperature is likely to be more deleterious than more rapid cooking at a high one. In experiments in which cabbage was heated at temperatures from 100° to 130° C. the destruction of antiscorbutic properties though extensive was less complete than would have been expected from the results at lower temperatures indicating the relatively low temperature coefficient of the destructive process above 100° as well as below.

The prolonged cooking of vegetables in a fireless cooker would thus be more destructive of vitamin C than actual boiling for a shorter time. Contrary to the results of Holst and Frölich with cabbage and of Harden and Zilza with lemon juice, the addition of dilute citric acid to the cabbage before boiling did not improve the resistance of the cabbage to the destructive action of heat. In view of these results and previous observations of the instability of vitamin C to alkalinization the author concludes that in cooking vegetables it is better to add neither acid nor alkali to the water in which they are boiled.

Delf and Skelton (1918) in experiments similar to those just described, found a loss in antiscorbutic potency of more than 93 per cent when cabbage was dried at a low temperature (60° C.) and stored subsequently for two to three weeks at laboratory temperatures. This loss increased with prolonged storage until at the end of three months nearly all the protective value of the fresh material was lost. By plunging the cabbage into boiling water before drying the residual amount of antiscorbutic factor was distinctly greater, indicating that killing the cells by heat before drying is beneficial in lessening the amount of destruction taking place during drying.

Givens and Cohen (1918) found the losses of vitamin C in drying to be much more serious for cabbage than for tomato.

Campbell and Chick (1919) studied the antiscorbutic and growth-promoting value of canned vegetables with special reference to the vitamin C content. The experiments consisted essentially in estimating and comparing the minimal amounts of the vegetables studied (cabbage and string beans) when raw and after being canned, which must be added daily to a basal scurvy-producing diet in order to protect young guinea-pigs from scurvy over a period of three months. The vegetables were washed in cold water, blanched, cold dipped and packed into lacquered cans, which were then nearly filled with boiling water, hermetically sealed and sterilized by exposure to steam at 100° C. for one and one-half hours in the case of cabbage, and on two successive days, or a total period of two and one-half hours in the case of the beans. The cabbage was tested two weeks, and the beans three months after canning. The conclusions drawn from this study are summarized as follows:

"In the process of canning vegetables the greater part of the original antiscurvy value of the raw vegetable is destroyed. In the case of runner bean pods (string beans) the loss is estimated at about 90 per cent of the original value; in the case of cabbage at about 70 per cent of the original value. . . . This loss is primarily due to the destruction of antiscurvy material occurring during the heating involved in the process of canning. A further loss may be expected to take place during the period of storage."

Delf (1920) reported a study of the effect of heating upon the antiscorbutic values of the juices of the cabbage, the "swede" (Swedish turnip), and the orange. When fed to guinea-pigs with a basal ration of oats, bran, and 60 to 90 cubic centimeters daily of autoclaved milk (the latter furnishing a little antiscorbutic vitamin) the minimal daily dose of the raw juice for the adequate protection of young guinea-pigs was about 1 cubic centimeter of cabbage, 2.5 cubic centimeters swede, and 1.5 cubic centimeter orange juice, respectively. On heating the juices to 100° C. for an hour the orange juice did not show any marked deterioration of antiscorbutic properties, while it required twice the dose of the raw juice in the case of the swede, and at least 7.5 times the amount of the raw juice of the cabbage to give the necessary protection. Only at 130° was any definite deteri-

oration in the orange juice detected, about twice as much being required as of the raw juice. In Delf's opinion, the differences in stability to heat of the three juices did not appear to be due to their hydrogen ion concentration.

The growth curves recorded in these experiments furnish evidence that growth is affected by the limitation of the antiscorbutic factor in the diet apart from the appearance of definite symptoms of scurvy and apart from deficiency in the other vitamins.

The stability of the swede and orange juice at temperatures above 100° was thought by the author to be due possibly to the absence of air during the process of heating in the autoclave. Later work has shown clearly that the antiscorbutic vitamin is sensitive to oxidation so that heating in air must be expected to be more destructive than heating at the same temperature for the same time in absence of air. Yet it would be a mistake to forget (as some writers now, 1921, seem inclined to do) that important losses of vitamin C occur through heat destruction in practical absence of oxygen (or even in presence of hydrogen) as well as through oxidation. The destruction of antiscorbutic vitamin during the drying of cabbage was not prevented by carrying out the process in an atmosphere of nitrogen (Ellis, Steenbock and Hart, 1921) nor was the destruction of about one-half the vitamin C which occurs on boiling tomato for one hour prevented by bubbling hydrogen through the solution. (La Mer, 1921.)

Seeds and Seed-pods.

Sound mature seeds, whether cereals or legumes, are either entirely devoid of vitamin C or contain so little of it as to show no antiscorbutic value in experiments upon the guinea-pig. However, with the sprouting of the seed a development of antiscorbutic property occurs which must mean that the resting seed contained some substance so closely related to vitamin C as to be transformed into it in the process of germination of the seed. Fürst (1912), working in the laboratory of Holst and Frölich, demonstrated this fact which under war conditions was confirmed both by feeding experiments upon guinea-pigs (Chick and Hume, 1916, 1917) and by successful application in the cure of human scurvy (Wiltshire, 1918).

Wiltshire (1918) compared germinated beans with lemon juice as antiscorbutic for scurvy patients (Serbian soldiers) in a war hospital in London. Thirty patients were treated with lemon juice and twenty-seven with germinated beans, the two groups of patients having been selected as being in as nearly the same condition as possible. Those treated with lemon juice received 4 ounces of the fresh juice daily. In comparison with those each patient in the other group received as his daily allowance of antiscorbutic the product resulting from 4 ounces of beans weighed dry, soaked 24 hours, germinated for 48 hours at a temperature of 60° to 70° C. and then cooked for 10 minutes. Of the patients receiving the germinated beans, 70 per cent recovered within four weeks as against 53 per cent of recoveries within the same length of time among those receiving the lemon juice. A better standard of comparison in Wiltshire's report was the time required for the return of the gums to normal condition, which averaged 3.1 weeks for the patients receiving germinated beans and 3.4 weeks for those receiving lemon juice. Judged from either standpoint it is evident that in this case the germinated beans were fully as efficient as the lemon juice in the quantities used.

Chick and Delf (1919) tested the antiscorbutic value of germinated peas and lentils which had been soaked in water for 24 hours, and germinated for 48 hours at room temperature. These were fed in comparison with the dry peas and lentils to guinea-pigs. Like Holst and Frölich, Chick and Delf found that the dry legumes have some antiscorbutic value since scurvy developed only after a longer interval and in a less acute form with these seeds than with cereals. The antiscorbutic value was estimated to be increased from 5 to 6 times by germination and the germinated peas and lentils were considered to compare favorably with many fresh vegetables in antiscorbutic value. A daily ration of 10 grams of the fresh germinated seeds equivalent to 5 grams of dry weight was found sufficient to prevent scurvy in guinea-pigs. Germinated oats and barley were also shown to have considerable antiscorbutic value. McClendon, Cole, Engstrand and Middlekauff (1919) have also reported experiments demonstrating the antiscorbutic value of germinated barley.

In the memorandum on the *Importance of Accessory Factors in the Food* issued by the British Committee on Accessory Food

Factors in June, 1919, for the guidance of those engaged in the administration of food relief to famine-stricken districts, the use of germinated seeds in the prevention of scurvy is given prominence. "If fresh vegetables or fruit are scarce or absent, an anti-scorbutic food can be prepared by moistening any available seeds (wheat, barley, rye, peas, beans, lentils) and allowing them to germinate. . . . The seeds should be soaked in water for 24 hours and kept moist with access of air for from one to three days, by which time they will have sprouted. This sprouted material possesses an antiscorbutic value equal to that of fresh vegetables and should be cooked in the ordinary way for as short a time as possible." The British Committee points out that the legume dhall is a staple Indian article of food, and had it been known that this could be made an effective antiscorbutic through simple sprouting, the great losses and suffering due to scurvy among Indian troops in the Mesopotamian campaign might have been avoided.

Seed-pods have been but little studied, though by analogy to fruits it might be expected that they should show antiscorbutic properties in the succulent stage of their development. The *cucumber* enjoys the reputation of a good antiscorbutic in Russia but does not seem to have been tested by modern methods. The immature seed-pod of the *string bean* has been found comparable with leaves and fruits in its content of vitamin C. (Campbell and Chick, 1919; Sherman and Campbell, unpublished data.) Since the string bean with its immature seed and the fleshy pod which feeds it are thus relatively rich in vitamin C, which disappears as the seed matures but appears again when the seed sprouts, it is plainly suggested that in the mature resting seed there must be present something which plays the part of a "resting stage" or reserve form of the antiscorbutic vitamin—a substance into which vitamin C is transformed as the seed matures and goes into the resting stage, and which in turn is transformed into vitamin C when the seed sprouts. The suggestion that vitamin B may be thus related to vitamin C has already been noted. Quantitative experiments to test this suggestion are much to be desired.

In this connection it will also be of interest and importance to determine whether the soaking of seeds to such an extent as sometimes precedes the application of heat in cookery, may

result in the development of any appreciable antiscorbutic power. As indicated above, Chick and Delf (1919) found evidence of slight antiscorbutic effects from soaked, but not visibly sprouted, legumes. And among the Russian peasants there is a belief that their black bread prepared from whole grain flour (including the embryo) by a slow process in which there is long soaking and an opportunity for considerable enzyme action to take place, has some value as an antiscorbutic, while white bread, made from the endosperm only and by a quicker process, has none.

Simple infusions of malted (sprouted) grains may possess sufficient antiscorbutic vitamin to be an important factor in the prevention of scurvy, as is indicated by experience as early as that of Captain Cook and as late as that of the South African negro labor camps in France during the World War (Hess, 1920); but Harden and Zilva (1918e) have shown that modern beer is quite devoid of antiscorbutic value.

Vitamin C in Animal Tissues and in Milk.

In the work of Parsons (1920) previously cited and in other investigations it has been made clear that the *liver* may be much richer in vitamin C than the *muscles*. Thus Parsons found that the livers of rats which had been for a long time on "scorbutic" food contained enough antiscorbutic vitamin to be readily demonstrable in experiments with guinea-pigs, whereas their muscles did not, nor could any antiscorbutic effect be obtained in parallel experiments in which fish muscle was fed.

Blood probably also carries more vitamin C than does muscle. Hess (1920) suggests that blood may be comparable with milk in its vitamin C content. This is not a sufficiently high concentration to make blood transfusion an important means of supplying the vitamin to a scurvy patient (Hess, 1920, p. 76). In fact the body as a whole seems to have only a very limited capacity for storage of vitamin C. Previous liberal feeding with antiscorbutic food does not enable guinea-pigs to survive materially longer when subsequently placed upon a scorbutic diet than do animals which have received merely an ample amount for the maintenance of health (Harden and Zilva, 1918b; Hess, 1920, p. 75). This is not inconsistent with the fact that a man or animal whose food has been deficient in vitamin C and who

is therefore already in a condition of latent scurvy may develop the disease more quickly when placed upon a wholly scorbutic diet than does an individual whose previous food supply has been normal. It is evident that we must look to the daily food supply rather than to any stores carried in the body for the antiscorbutic vitamin needed in nutrition.

Muscle tissues, ordinary *meats*, are so poor in antiscorbutic vitamin that attempts to show its presence by experiments upon guinea-pigs have given negative results. Chick, Hume and Skelton (Hess, 1920, p. 168) found that 10 cubic centimeters daily of raw beef juice failed to protect guinea-pigs. Dutcher, Pierson and Biester (1919) were not able to observe any antiscorbutic effect from raw lean beef fed to guinea-pigs. On the other hand, observations upon human scurvy have sometimes indicated that meat, if eaten sufficiently fresh, raw or "rare," and in large quantities, has an appreciable though small antiscorbutic value. Thus the fresh beef and horse meat eaten by the British troops in Mesopotamia is believed to have been largely responsible for the fact that they did not develop scurvy as did the Indian troops serving with them. In 1877 the British Arctic Survey Committee reported in regard to the outbreak of scurvy in the Polar expedition of 1875-76, that "although the scurvy was due to the absence of lime juice from the sledge dietaries, meat in large amounts is able to prevent the disease." More recently Stefánsson (1918, 1918a) has reported avoidance of scurvy during his Arctic explorations by the use of large quantities of meat from freshly killed game, this meat being usually eaten raw. In view of the fact that even when eaten in large amounts meat can be expected to prevent scurvy only if eaten raw or nearly so, we must conclude that cooked meat, as ordinarily eaten, probably furnishes but insignificant amounts of the antiscorbutic vitamin.

Milk is a much more important source of antiscorbutic vitamin than is meat, though as recent experiments have shown, the amount of vitamin C in milk may vary considerably with the food of the cow. Probably because of this possibility of variation some writers speak cautiously of dependence upon milk for vitamin C, while Rosenau (1921) emphasizes the statement that "Milk is rich in all of the known vitamins."

While the quantity of milk required to furnish the necessary

amount of vitamin C is much larger than the quantity of orange, lemon or tomato juice, so that it is not rich in vitamin C in the same sense as these latter foods, yet Rosenau's characterization of milk as rich in all the known vitamins is true in the sense that with fresh milk as the sole or chief food, all of the vitamins would ordinarily be supplied in abundance. With milk as with potatoes it is not so much the concentration of the vitamin C in the food as the quantity in which it normally is, and advantageously can be, consumed which makes it important as an antiscorbutic. Several experiments designed to determine the quantity of milk required for prevention of scurvy in the guinea-pig have been reported.

Chick, Hume and Skelton (1918) found that with milk as sole source of vitamin C, complete protection was secured when guinea-pigs were fed an average of 85 cubic centimeters of milk per capita per day. On the usual assumption that a baby requires about five times as much vitamin C as a guinea-pig, this result agrees well with the general experience of Hess (1920, p. 152) that an infant will receive sufficient antiscorbutic vitamin from one pint of fresh milk per day. These estimates apply to milk of average or nearly average antiscorbutic value. If the antiscorbutic value is materially lessened by heating or aging of the milk or by faulty feeding of the cow, more milk may be required; and conversely fresh milk from a cow properly fed will usually be somewhat richer and may be considerably richer in vitamin C than these estimates would indicate.

Hart, Steenbock and Smith (1919) reported studies of experimental scurvy in guinea-pigs the results of which confirmed the above conclusions on the quantitative relation of raw milk consumption to the development of scurvy. In addition, evidence was presented that their samples of commercial evaporated milk, commercial milk powder, and milk sterilized for 10 minutes at 120° C. did not show antiscorbutic properties when used in quantities equivalent to an amount of raw milk which would prevent scurvy in guinea-pigs on a diet of rolled oats and dried hay. Since it is now evident that the feed of the cow has much influence upon the antiscorbutic value of milk, negative results of this character must be accepted only for the conditions actually covered by the experiments.

Canned milks differ greatly as regards their antiscorbutic

value, not only because the concentration of vitamin C in the original milk is subject to variation with the feeding of the cow as above mentioned, but also because the canning process involves very different heat treatment in different cases. Unsweetened canned milk (the "evaporated" milk of commerce) depends for its preservation upon concentration and heat treatment only and is given such rigorous heating to ensure against subsequent spoilage that the antiscorbutic vitamin is apt to be almost entirely destroyed (Hart, Steenbock and Smith, 1919). On the other hand, milk canned with sugar (commonly called sweetened condensed, or commercially simply "condensed" milk) is preserved in large measure by the sugar present as well as by the concentration of the milk solids, and therefore needs less heat treatment. Such condensed milk Hess (1921) finds "to retain the larger part of its antiscorbutic factor," and it has also been found by Hume (1921a) to contain its antiscorbutic vitamin in apparently undiminished amount.

Milk scalded by being heated rapidly to the boiling point over a gas burner and then quickly cooled was found by Barnes and Hume (1919) to retain about one half of its original antiscorbutic value.

It seems probable that on the average there is also a loss of something like one-half of the original antiscorbutic value when milk is dried by present-day methods. The report of Coutts (1918) to the Local Government Board stating on the basis of experience in England, Belgium and France "that scurvy is not to be feared" from the use of dried milk in infant feeding, is favorably quoted by Hess (1920) who states that in his own experience "dried milk not only does not lead to scurvy, but may contain sufficient antiscorbutic vitamin to cure this disorder." Hess attributes the unfavorable results obtained by Hart, Steenbock and Smith (1919) with dried milk to lack of uniformity in the product and suggests that "For milk to retain its antiscorbutic value, notwithstanding drying, it must have been rich in vitamin before desiccation, it must have been dried quickly, and packed within the shortest possible interval in air-tight, preferably hermetically sealed, containers. As in relation to the heating of milk, so in regard to its drying, it is not the degree of heat to which it is subjected which is all-important, but rather the associated conditions. The merits of each process will have to

be tested individually and perhaps even each particular brand of milk."

In the work of Barnes and Hume above cited some evidence was obtained indicating that winter milk was inferior to summer milk in antiscorbutic properties, corresponding to the differences in the cow's diet at these different seasons. In this connection the suggestion was made that the value of winter milk in this respect might be raised if swedes were employed for winter feeding in place of mangolds.

Hart, Steenbock and Ellis (1920) studied the influence of the feed of the cow upon the antiscorbutic value of her milk. The samples of milk tested included dry feed milk, obtained from a herd of 18 cows which had never been fed any fresh vegetable tissues but only air-dried roughages and grains; summer pasture milk from cows which during part of the day grazed on a timothy, blue grass, clover pasture; and winter-produced milk from cows fed on dried grains and hays, supplemented in one case by a corn silage made from corn that had well matured and partly dried but had not been frozen, and in another case by a small amount of silage and a considerable amount of hybrid sugar mangels. Each variety of milk was tested for its antiscorbutic vitamin content by feeding it to guinea-pigs in amounts varying from 15 to 50 cubic centimeters per animal in the case of the summer pasture milk and from 15 to 100 cubic centimeters in the case of the dry feed milks, the milk in each case serving as a supplement to a basal scurvy-producing ration of heated ground alfalfa hay, rolled oats, and common salt.

A daily consumption of 15 cubic centimeters of summer pasture milk afforded protection against scurvy for 20 weeks to one guinea-pig but did not protect completely two others in the same group. On increasing the amount to 30 cubic centimeters two animals out of three were protected, the third developing scurvy in 8 weeks. Full protection was secured by the daily consumption of 50 cubic centimeters of the milk.

In the case of the dry feed milk 15 cubic centimeters and 30 cubic centimeters daily completely failed to prevent scurvy, 50 cubic centimeters delayed the onset but did not afford entire protection, and 75 cubic centimeters furnished complete protection. The fact that protection against scurvy was ultimately secured with the dry feed milk indicates that the materials fed,

although dried and at least a year old, still had some antiscorbutic potency.

Both varieties of the winter milk above mentioned proved only slightly richer in the antiscorbutic vitamin than the dry feed milk, complete protection not being secured until 75 cubic centimeters of milk daily had been added to the basal ration. The sugar mangel milk was a slightly better source for this vitamin than the silage milk.

Dutcher, Eckles, Dahle, Mead and Shaeffer (1920) have experimented somewhat similarly but with the same cows, first on winter and then on summer feed. They conclude that 20 cubic centimeters of summer milk were superior to 60 cubic centimeters of winter milk in antiscorbutic value. It was found that there was a tendency for the milk to become poor slowly when the diet of the cow is low in vitamin, while the milk tends to become rich rapidly, so far as vitamins are concerned, when the cow is placed upon a good vitamin-rich diet.

Hess, Unger and Supplee (1920) have also shown that there is much more antiscorbutic vitamin in the milk of cows on pasture than in that of those receiving a ration believed to be otherwise adequate but nearly devoid of antiscorbutic substances.

Thus it appears fully demonstrated that the diet of the milk-producing animal is a very large factor in the relative antiscorbutic potency of the milk produced, some samples of milk having at least three times as much antiscorbutic vitamin as others when the only variable factor of importance appears to be the food of the cow.

We have seen that foods of high initial acidity such as tomatoes and orange and lemon juice can be dried and still retain marked antiscorbutic value while attempts to conserve vitamin C during the drying of less acid foods such as cabbage and potatoes have been less successful. The drying of milk is perhaps more highly developed from the mechanical standpoint than that of any other food and since it is nearly neutral in reaction and largely used in infant feeding, special interest attaches to the question of retention of the antiscorbutic value of milk during drying. Some tests of milk dried by various commercial processes have shown antiscorbutic value and others have not (Hess, 1920; Hart, Steenbock and Ellis, 1921; Jephcott and Bacharach, 1921). The tendency has been to ascribe the

difference to the mechanical process used in drying, but in view of the fact that the initial concentration of vitamin C in milk is now known to vary rather widely, and the further fact that the mechanical devices for the drying of milk are undergoing still further development, it is doubtful if the differences reported should be attributed to the mechanical process alone. The time and temperature of heating to which the milk is subjected in drying depend not only upon the mechanical principle of the process but also upon the manner in which it is carried out.

In our opinion it would be unfortunate if the development of the methods for the drying of milk should be prejudiced by too great an emphasis upon one or another mechanical feature at the present time.

Reported Occurrence of Vitamin C.

Food Material.	*Literature Reference.*
Almonds (sprouted)	Fürst 1912.
Apple	Holst and Frölich 1912; Chick and Rhodes 1918; Robison 1919; Givens, McClugage and Van Horne 1921.
Banana	Holst and Frölich 1912; Lewis 1919; Sugiura and Benedict 1919; Givens, McClugage and Van Horne 1921.
Barley (sprouted)	Holst, 1912; Cohen and Mendel 1918; McClendon, Cole, Engelstrand, and Middlekauff 1919.
Beans (sprouted)	Fürst 1919; Wiltshire 1918.
green string	Campbell and Chick 1919.
Cabbage	Holst and Frölich 1912, 1913, 1916, 1920; Cohen and Mendel 1918; Givens and Cohen 1918; Delf 1918, 1920;

Food Material.	*Literature Reference.*
Cabbage	Delf and Skelton 1918; Campbell and Chick 1919; Ellis, Steenbock and Hart 1921.
Carrots	Holst and Frölich 1912; Chick and Rhodes 1918; Cohen and Mendel 1918; Hess and Unger 1919; Shorten and Roy 1919; Zilva 1920; Hess 1920.
Cauliflower	Holst and Frölich 1912.
Cloudberries	Holst and Frölich 1912.
Cocum (dried)	Chick, Hume and Skelton 1919.
Dandelion	Holst and Frölich 1912.
Endive	Holst and Frölich 1912.
Grape juice	Chick and Rhodes 1918.
Grapefruit juice, dried	Givens and Macy 1921.
Legume (sprouted)	Chick and Hume 1916-17, 1917.
Lemon juice	Holst and Frölich 1912; Chick, Hume and Skelton 1918a; Smith 1918; Harden and Zilva 1918a; Wiltshire 1918; Harden, Zilva and Still 1919; Stevenson 1920; Davey 1921; Zilva 1921.
juice tablets juice dried	Bassett-Smith 1920, 1920a, 1921. Givens and Macy 1921.
Lentils (sprouted)	Fürst 1912; Greig 1917; Chick and Delf 1919.
Lettuce	Holst and Frölich 1912.

Food Material.	*Literature Reference.*
Lime juice	Holst and Frölich 1907; Frölich 1919; Fürst 1912; Chick, Hume and Skelton 1918b; Smith 1918; Robison 1919; Davey 1921.
Malt, green	McClendon and Cole 1919.
—extract	McClendon, Cole, Engstrand and Middlekauff 1919.
soup extract	Gerstenberger 1921.
Mango dried	Chick, Hume and Skelton 1919.
Meat juice raw	Wilcox 1920.
Milk, whole raw	Frölich 1912; Funk 1913; Chick, Hume and Skelton 1918; Cohen and Mendel 1918; Barnes and Hume 1919; Hart, Steenbock and Smith 1919; Hess and Unger 1919a; Hart, Steenbock and Ellis 1920; Dutcher, Eckles, Dahle, Mead and Schaeffer 1920; Hess 1920; Hess and Unger 1921.
pasteurized	Frölich 1912; Hess and Fish 1914; Barnes and Hume 1919; Anderson, Dutcher, Eckles and Wilbur 1921.
boiled	Anderson, Dutcher, Eckles and Wilbur 1921. Nobel 1921.
sterilized 120° C. 10 minutes	Hart, Steenbock and Smith 1919;
dried	Coutts 1918; Winfield 1918;

Food Material.	*Literature Reference.*
Milk, dried	Barnes and Hume 1919; Hart, Steenbock and Smith 1919; Hess and Unger 1919a; Hess, Unger and Supplee 1920; Hart, Steenbock and Ellis 1921; Jephcott and Bacharach 1921.
sweetened condensed	Hess 1921; Hume 1921a.
lactic acid milk	Stevenson 1920.
Mongo (sprouted)	Santos 1921.
Mulberries	Holst and Frölich 1912.
Oats (sprouted)	Cohen and Mendel 1918.
Onions	British Committee Report 1919. Shorten and Roy 1919.
Orange	Harden and Zilva 1918, 1920; Cohen and Mendel 1918;
juice	Hess 1918; Cohen and Mendel 1918; Hess and Unger 1918a, 1919; Delf 1920; Ellis, Steenbock and Hart 1921; Davey 1921.
juice dried	Harden and Robison 1919, 1920; Givens and McClugage 1919a; Harden and Robison 1921; McClendon and Dick 1921; McClendon, Bowers and Sedgwick 1921.
Orange peel	Hess 1918; Hess and Unger 1918a; Hess 1920; Cooper, E. 1921.
Peas (sprouted)	Fürst 1912; Greig 1917; Chick and Delf 1919; Stevenson 1920.

Food Material.	*Literature Reference.*
Potatoes	Holst and Frölich 1912; Chick and Rhodes 1918; Givens and Cohen 1918; Hess 1920; Bezssonoff 1921, 1921a.
dried	Givens and McClugage 1920a; Shorten and Roy 1921.
Raspberries	Holst and Frölich 1912.
Rhubarb	Pierson and Dutcher 1920.
Rutabaga (Swede)	Chick and Rhodes 1918; Delf 1920.
Seeds (sprouted)	Chick and Hume 1916-17, 1917.
Sorrel	Holst and Frölich 1912.
Spruce infusion	Appleton 1921a.
Tamarind dried	Chick, Hume and Skelton 1919.
Tomatoes	Hess and Unger 1918, 1919, 1919a. Hess 1920; Eddy and Stevenson 1920; La Mer, Campbell and Sherman 1921; Bassett-Smith 1921; Givens and Macy 1921.
dried	Givens and McClugage 1919, 1919a, 1921; Shorten and Roy 1921.
Turnip	Holst and Frölich 1912.
Turnip, Swedish	See Rutabaga.

Quantitative Determination and Study of Chemical Behavior.

As Hess points out (1920, p. 173) "For well over a hundred years it was generally known that scurvy could be cured by fruits and vegetables, and yet no further progress was made toward a more complete understanding of the value of these foodstuffs. . . . Decided advance has been made only in the past few years since these foodstuffs have been studied from the quantitative viewpoint."

The beginnings of our modern knowledge of the quantitative determination of antiscorbutic values, as of so many other aspects of scurvy and the antiscorbutic vitamin, are to be found in the work of Holst and Frölich (1912), who made comparisons of the antiscorbutic values of foods by determining whether or not certain empirically chosen quantities of foods which they fed would suffice to prevent the appearance of scurvy in guinea-pigs. They tabulated fully the results of experimental feeding with and without the antiscorbutics and made some progress toward the standardization of the test animals, but in general they fed the foods to be tested only at one level of intake, and the basal diet which they employed, consisting usually of oats and water only, was deficient in other respects as well as in antiscorbutic vitamin.

Cohen and Mendel (1918) devised a new basal ration in the form of a "soy bean cracker" made of cooked soy bean flour, cellulose, sodium chloride, calcium lactate, dried brewer's yeast, and milk, planned to provide all necessary nutrients except the antiscorbutic. They showed that experimental scurvy could be induced in the guinea-pig at will, and formulated more fully the criteria for the recognition of this condition.

Hess and Unger (1918a) employed chiefly a basal ration of oats, hay and water. This has also been used in some of the recent work in other laboratories, alfalfa hay being especially advocated.

Chick, Hume and Skelton, Delf, and other workers at the Lister Institute have used a basal diet of oats and bran *ad libitum* with a liberal allowance (usually 60 cubic centimeters per guinea-pig per day) of milk autoclaved at 120° for one hour. This addition of milk to the basal diet was found to result in a much better general condition of the animals as would be expected in view of the extent to which the diet is thus improved in its mineral and vitamin A content, and the nutritive efficiency of the protein mixture which the combination of grain and milk affords. As Hess points out, however (1920, p. 117), this milk still retains a small amount of antiscorbutic vitamin; and from the results of other work we know that the amount of vitamin C, thus introduced into the basal diet which should be free from it, while small, is variable and therefore must detract from the quantitative accuracy of work in which this basal ration is used.

Building upon the experience of previous investigators as well as upon the results of their own studies of the above mentioned basal diets and several modifications of them, Sherman, La Mer and Campbell (1922) have still further developed the basal ration to ensure its freedom from vitamin C and its entire adequacy in all other respects.

The basal diet as finally adopted is as follows:

Oats, sound whole grain, ground in the laboratory as needed	59%
Skimmed milk powder, heated on open trays at 110° C. until all antiscorbutic vitamin is destroyed	30%
Butter fat, freshly prepared	10%
Sodium chloride	1%
	100%

This diet supports excellent growth up to the time of the onset of scurvy.

Sound mature oats show no antiscorbutic property when fed to guinea-pigs and are eaten readily by them. By using heated skimmed milk and fresh butter fat instead of heated whole milk, the absence of antiscorbutic vitamin is at least equally well ensured and the palatability of the diet and its fat-soluble vitamin content are improved. The heat treatment necessary to ensure complete destruction of vitamin C in the skimmed milk powder should be determined by each investigator for his own material and technique, keeping in mind the likelihood of variation in antiscorbutic vitamin content of milk with the season and the importance of eliminating this vitamin completely from the basal ration without subjecting the food to such excessive heating as would give it a burnt taste and prevent its being eaten readily by the experimental animals. In our experiments, two hours heating at 110° C. in shallow trays freely exposed to the air of the oven was found to be sufficient, as determined by controlled feeding experiments. After such heating the milk powder is of a light buff color.

The butter fat, prepared by melting butter of good quality at the lowest possible temperature and freeing from water and curd

by decantation and filtration through paper, is intimately mixed with the heated milk powder, the salt, and the freshly ground oats, so that the constituents of the mixture can not be separated by the experimental animal while eating. The food mixture should be kept in a refrigerator in well-filled, air-tight containers; fresh portions fed daily, and feeding cups thoroughly cleansed at least twice a week. Even the slightest incipient rancidity may result in failure of the animals to eat the food readily, in which case the interpretation of results will be difficult and probably of very doubtful value.

Selection and Care of Experimental Animals.

The experimental animals should either be bred by the investigator or purchased at an early and known age. They should be known to be growing at a normal rate. For about a week before the beginning of the experimental period they should be housed in the experimental cages or pens and fed with the above basal ration plus green food, both *ad libitum*. The experiment proper is then begun by simply discontinuing the green food with or without the feeding of a measured amount of antiscorbutic in addition to the basal ration. Guinea-pigs six to eight weeks old and weighing 300 to 350 grams are best used for this purpose. Placed at this age and size upon the above basal diet with food and water always available they usually eat about 18 to 20 grams of the dry food mixture per day and continue to grow for about 15 days, then lose weight rapidly and die of scurvy in from 26 to 34 days after being deprived of antiscorbutic food. If the animals are much younger, the results are somewhat less regular; if much older, they are somewhat less susceptible, and also less likely to show good growth up to the time of onset of scurvy symptoms.

Symptoms, Survival Period, and Autopsy Findings.

With animals such as have been described, the first symptoms of scurvy appear after about 12 days on the above basal diet. The nature and sequence of the symptoms has been so fully and clearly described by Cohen and Mendel (1918) and by Hess (1920, pp. 135-140) as not to require detailed discussion here. Loss of weight usually begins soon after the appearance of the

first symptoms, though some animals begin to lose weight earlier. On the first or second day of the experimental period there may occur a decrease of body weight due to elimination of bulky intestinal contents from the green food of the fore-period. In such cases the minimum weight of the first or second day is taken as the initial weight of the experimental period. As an accurate weight curve is helpful in the interpretation of results, each animal should be weighed at least once in three days throughout the experimental period. Fig. 6 shows the average weight curve of 10 guinea-pigs kept on the basal diet above described. Since the time of reaching a maximum and beginning to lose weight differs with individuals, the effect of averaging the results is to blunt the typical peak of the weight curve. For this reason there is also given in Fig. 6 the weight curve of an individual guinea-pig which shows in pronounced degree the initial fall in body weight upon placing on the basal diet, followed by rapid growth in weight to a sharp maximum, then followed by rapid loss of weight after the onset of scurvy. The weight curves in the majority of cases may be expected to be intermediate in character between the two curves shown in Fig. 6.

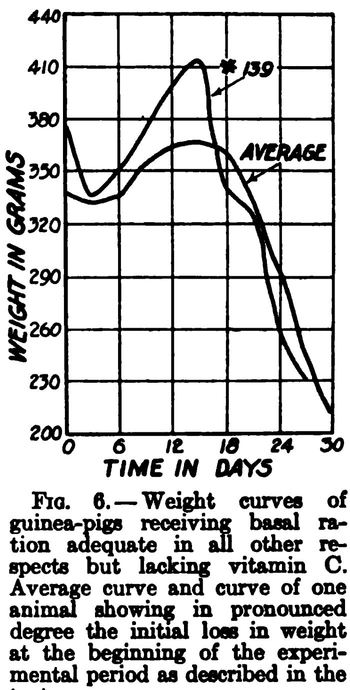


FIG. 6.—Weight curves of guinea-pigs receiving basal ration adequate in all other respects but lacking vitamin C. Average curve and curve of one animal showing in pronounced degree the initial loss in weight at the beginning of the experimental period as described in the text.

The survival periods of 15 successive animals kept upon the above basal diet only were as follows: 33, 26, 27, 28, 34, 28, 27, 34, 26, 29, 32, 32, 32, 31, 34 days. At autopsy the findings which proved most significant were: looseness of teeth, fragility of bones, enlargements and hemorrhages of joints and rib junctions. For full description of the pathology of experimental scurvy the reader is referred to the monograph of Hess (1920, pp. 122-125, 129-130.)

Quantitative Rating of Severity of Results.

The results obtained by Sherman, La Mer and Campbell (1922) on feeding the above-described basal ration alone and with the addition of filtered juice of canned tomatoes in measured amounts calculated to the basis of 300 grams of guinea-pig were in brief as follows:

With no antiscorbutic there is good initial growth followed by onset of scurvy symptoms, cessation of growth and great loss of weight before death from scurvy which occurs at 26 to 34 days. Autopsy reveals in severe form all the typical signs of scurvy, notably hemorrhages, fragility of bones, and looseness of teeth. (See table below.)

With 1.0 cubic centimeter tomato juice per day the duration of life is prolonged and becomes less uniform than on the completely scorbutic basal diet. The animals become lame and stiff before death and at death show severe hemorrhages, fragile bones and loose teeth.

With 1.5 cubic centimeters tomato juice the animals usually live out the experimental period of 70 to 90 days after which it is unlikely that death from scurvy will occur. Scurvy symptoms develop with pain and stiffness in the joints and usually with loss in body weight. Hemorrhages and enlargements of rib junctions may become quite as pronounced as in the previous cases. (Since the animals live longer there is more time for these abnormalities to develop.) Fragility of the bones and looseness of teeth are less marked than when less antiscorbutic is given.

With 2.0 cubic centimeters tomato juice growth after 15 days is subnormal and animals show soreness of joints without noticeable stiffness. When the animals are killed and examined after 70 to 90 days on this diet they show hemorrhages but not to a pronounced degree. Jaws and teeth appear normal and bones usually so.

With 3.0 cubic centimeters or more of tomato juice there is complete protection from scurvy as judged by examinations both during life and at autopsy. Growth is fully normal in all animals that eat the basal diet well.

The accompanying table shows the protocols of typical cases of guinea-pigs kept on the basal diet alone or with measured amounts of tomato juice as antiscorbutic. In all cases the

volume of tomato juice as stated in the table is the amount which was fed per 300 grams guinea-pig. This reduction of the dosage of antiscorbutic to a uniform basis of body weight of standard animal has been found to add considerably to the quantitative significance of the results. Following the precedent of Holst and Frölich the severity of autopsy findings such as hemorrhages and fragility of bones and looseness of teeth are indicated by — (no different from normal), ? (doubtful), tr (trace), and +, ++, +++ for increasing degrees of severity. From this series of observations upon animals receiving no antiscorbutic vitamin and with different measured amounts up to the amount which affords complete protection and permits optimum growth it becomes possible to interpret the symptoms and autopsy findings in terms of the percentage of the required amount of antiscorbutic which was actually received by the animal in any individual case. This means that animals fed on this basal diet and receiving some antiscorbutic but not enough for complete protection can be given a quantitative rating based on the weight curve, survival period, and severity of the symptoms and autopsy findings. Thus, in comparing the antiscorbutic properties of different foods or of the same food before and after treatment, one is not confined entirely to a comparison of the so-called minimum protective doses since the quantitative rating of the degree of protection afforded permits comparisons to be made upon animals receiving less than the amount required for complete protection.

Quantitative Study of Chemical Behavior.

This method was applied in studying the heat destruction of the antiscorbutic vitamin quantitatively, with feeding experiments to determine how much more of the heated juice must be fed in order to get the same result as with a known quantity of raw juice. Comparison of the quantities of the two juices necessary to give the same degree of protection then enables one to calculate the percentage of antiscorbutic vitamin which had been destroyed by the heating.

The last four lines in the table give the results of experiments of that kind. Comparing the findings in the case of No. 155 with results given previously, it was considered that on the whole 3.9 cubic centimeters of tomato juice heated for one hour

PROTOCOLS OF EXPERIMENTAL ANIMALS ON BASAL DIET ALONE OR WITH ANTISCORBUTIC.

Animal No.	Tomato Juice c.c.	Body Weight: Initial grams	Body Weight: Maximum grams	Body Weight: Final grams	Duration of Experiment	Symptoms	Autopsy Findings — Bony System: Jaw	Bony System: Teeth	Bony System: Ribs	Bony System: Joints	Hemorrhages: Ribs	Hemorrhages: Intestines	Hemorrhages: Joints	Hemorrhages: Muscles
143 ♂	0.0	361	439	219	28 days	Very severe	+++	+++	+++	++	++	++	++	++
193 ♀	0.0	329	351	182	34 "	Very severe	+++	+++	+++	+++	+++	+++	+++	+++
207 ♂	0.0	321	350	192	28 "	Very severe	+++	+++	+++	+	+++	++	++	++
173 ♀	1.0	312	405	269	90* "	Very severe	+	—	+++	+++	+++	+	+	+
184 ♂	1.0	321	384	255	53 "	Very severe	++	++	+++	+++	+++	+	—	++
90 ♀	1.4	332	394	201	41 "	Severe	+	+	tr	tr	+	+++	—	tr
93 ♂	1.4	321	468	285	63 "	Severe	+	+	+++	+++	+++	+	+++	
92 ♂	1.5	332	368	285	91* "	Severe	tr	tr	++	+		+		tr
94 ♀	2.0	309	340	311	87* "	Moderate	—	—	+	++	—	+	++	tr
95 ♂	2.1	282	388	345	91* "	Moderate	—	—	?	+	—	—	+	—
155 ♂	(a)	320	402	383	73* "	Mild	—	—	+	+		tr	—	—
181 ♀	(b)	323	503	475	85* "	Mild	—	—	? tr	—	—	tr	—	—
199 ♀	(c)	337	505	492	85* "	Very mild	—	—	? +	—	—	+	—	—
130 ♂	(d)	337	415	390	73* "	Moderate	—	+	++	+	—	+	+	+

(a) Received 3.9 cc tomato juice which had been heated One hour at 100°C. judged equal to 2.0 cc. of raw juice.
(b) Received 7.0 cc tomato juice which had been heated Four hours at 100°C. judged equal to 2.5 cc of raw juice.
(c) Received 4.0 cc tomato juice which had been heated Four hours at 60°C. judged equal to 2.5 cc of raw juice.
(d) Received 2.3 cc tomato juice which had been heated One hour at 60°C. judged equal to 1.75 cc of raw juice.
* These animals were killed for autopsy; the others died of scurvy.

at 100° C. showed practically the same antiscorbutic effect as 2 cubic centimeters of the unheated tomato juice and therefore that approximately one-half of the antiscorbutic vitamin of the tomato juice had been destroyed by the heat treatment. The average of a considerable number of such experiments is practically 50 per cent.

In the next case (No. 181) the animal received 7 cubic centimeters of tomato juice heated at 100° C. for 4 hours. The results indicated that this amount of heated juice was equivalent in antiscorbutic value to 2.5 cubic centimeters raw juice, and the average of a number of such experiments indicated that heating for four hours at 100° resulted in the destruction of about 68 per cent of the antiscorbutic vitamin present in the raw juice. The results of experiments on Nos. 199 and 130 (notes "c" and "d") show results of heating at 60° with, of course, a lower rate of destruction than at 100°.

It is plain that in the same way one may compare the antiscorbutic potency of a measured amount of any other material with that of any of the different amounts of canned tomato juice representing any degree of protection up to the complete protection afforded by 3 cubic centimeters and can thus determine the relative amounts of antiscorbutic vitamin in different foods both from experiments in which the exact minimum protective dose is found and from those in which there is a definite partial protection to which a quantitative rating can be given. This method possesses the advantage of the method of minimum protective doses, and in addition permits the use of a numerical value for each individual experiment of a series, the average of which should yield a more trustworthy result than when only those animals receiving exactly the minimum protective dose are taken into account.

It is believed that the use of the method just described as a standard procedure in future studies of the antiscorbutic values of foods and of the behavior of vitamin C under different conditions will both facilitate the performance and interpretation of such experiments, and add greatly to the quantitative significance of the results obtained. The method has been used in a recent investigation (La Mer, Campbell and Sherman, 1921, 1922) of the effect of temperature and the concentration of hydrogen ions upon the rate of destruction of vitamin C. The

resulting time curves for destruction of the vitamin at 60°, 80° and 100° C. in aqueous medium of the acidity of natural tomato juice are shown in Fig. 7 and the chief results of the study may be briefly summarized as follows:

Effect of heating in acid solution. In the case of tomato juice of natural acidity, pH = 4.3, it was found that boiling for one hour destroyed practically 50 per cent, and for four hours practically 68 per cent of the antiscorbutic vitamin present. The

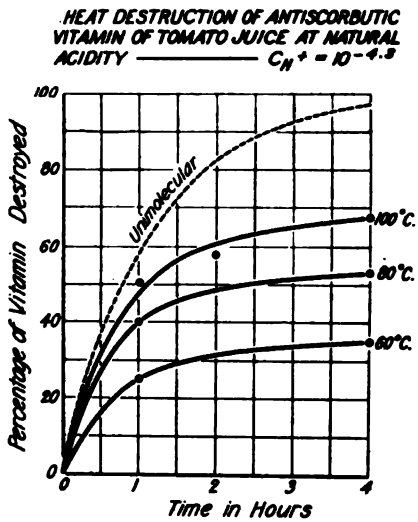


FIG. 7.—Heat destruction of antiscorbutic vitamin in tomato juice of natural acidity at 60°, 80° and 100° C. See text.

time curve of the destructive process is, therefore, much flatter than that of a unimolecular reaction or of a reaction proceeding according to the square root rule of Schütz. Similar flattening of the time curves of the destruction of the vitamin were found also in experiments in which the tomato juice was heated at temperatures lower than boiling, viz., at 60° and at 80°. Comparisons of the data obtained at 60°, 80° and 100° show relatively low temperature coefficients:

$$Q_{10}\ (60°—80°) = 1.23;\ Q_{10}\ (80°—100°) = 1.12.$$

Effect of reduced concentration of hydrogen ions. In experiments in which the natural acidity of the standard tomato juice was first neutralized in whole or in part, the juice then boiled

for one hour and immediately cooled and reacidified, it was found that at pH 5.1 to 4.9 (natural acidity less than half neutralized) the destruction during one hour's boiling was increased to 58 per cent (as compared with 50 per cent at natural acidity, pH = 4.3). Neutralization of a larger proportion of the natural acidity increased the rate of destruction of the vitamin. When alkali was added to an initial pH of 11, which fell to about 9 during the hour of heating (doubtless because of reaction of the alkali with the sugars present), the destruction found by feeding of the juice thus treated but immediately cooled and reacidified, was about 65 per cent.

In all of these experiments the heating was performed in cotton-stoppered, narrow-necked flasks from which air was probably very largely displaced by water vapor early in the heating. When the experiments upon heating at 100° were repeated with oxygen bubbling through the solution the destruction of the vitamin was much more rapid.

Heating at 100° for one hour at pH = 11 to 9 as described above, followed by standing for one to five days in stoppered but only partially filled bottles in a refrigerator at 10° C. at an alkalinity of only pH = 9, was found to destroy 90 to 95 per cent of the antiscorbutic vitamin, as compared with 65 per cent when the solution was reacidified after heating. This confirms the observations of Harden and Zilva (1918d) and of Hess and Unger (1919) upon the susceptibility of this vitamin to alkalinity even at low temperatures.

Thus while the great instability of the antiscorbutic vitamin makes it an unpromising material for attempts at actual isolation, the development of methods for its quantitative measurement makes possible the study of its chemical behavior.

The chemical behavior of vitamin C, especially as regards its susceptibility to oxidation, is under such active investigation at the present time (1921) that any attempt at summary is likely to be superseded while being written and printed. Five papers which have recently appeared have together shown that strong oxidizing agents such as potassium permanganate and hydrogen peroxide destroy vitamin C more or less rapidly even in the cold and that aëration at 100° C. rapidly destroys this vitamin when present in a medium such as decitrated lemon juice. (Hess, 1921; Zilva, 1921; Ellis, Steenbock and Hart, 1921; Anderson,

Dutcher, Eckles and Wilbur, 1921; Dutcher, Harshaw and Hall, 1921.) From this work some have gained the impression that the process by which vitamin C is destroyed is purely oxidative and that the sole effect of heat is to increase the rate of this oxidative reaction. Undoubtedly, however, heat destruction as such was the chief factor involved in the experiments of La Mer, Campbell and Sherman, above described, in which tomato juice at natural acidity lost 50 per cent of its antiscorbutic vitamin in one hour at 100°, and when rendered faintly alkaline lost about 61 to 65 per cent under the same heat treatment.

Hence it appears that vitamin C is quite susceptible to destruction both by oxidation and by heat treatment *per se*. It is quite possible that the heat destruction of vitamin C is of the nature of an intramolecular oxidation and reduction such as that of the Canizzaro reaction. The addition of an external oxidizing agent would then tend toward a more rapid and complete destruction of the vitamin as in the experiments mentioned above. Of interest in this connection is the fact that recent preliminary experiments by La Mer (1921a) indicate an increased rate of destruction on heating in the presence of hydrogen. If further work shall show that reduction as well as oxidation increases the rate of destruction of the vitamin, the finding will obviously favor the view that the vitamin is susceptible to destruction by a reaction of simultaneous oxidation and reduction as well as by oxidation in the ordinary sense. Further study of this problem from the standpoint of quantitative consideration of oxidation and reduction potentials and hydrogen ion concentrations should yield valuable results.

Summary of Physical and Chemical Properties of Vitamin C.

Vitamin C, the antiscorbutic vitamin, is freely soluble in water and in alcohol (Hess and Unger), dialyzes through parchment (Holst and Frölich), passes through a porcelain filter and is not adsorbed (as is vitamin B) by fuller's earth or Lloyd's reagent (Harden and Zilva). Despite this latter difference the similarity of solubilities between vitamins B and C and the fact that the latter apparently passes to and from an inactive substance in the ripening and subsequent germination of seeds (the

mature resting seed which lacks C, being particularly rich in B) have led to the suggestion that vitamins B and C may stand in close chemical relationship to each other—a suggestion however for which as yet the experimental evidence is but rudimentary. Vitamin C is much more readily destroyed than is vitamin B, both by oxidation and by heating *per se*.

The destruction of vitamin C by heating in water solution is a reaction which proceeds at a measurable rate and which has been studied quantitatively with reference to the influence of the factors time, temperature, and hydrogen ion concentration. (Delf; La Mer, Campbell and Sherman.) According to Delf, the antiscorbutic vitamin is more resistant to heat destruction when contained in the cells of the cabbage leaf than when in the expressed juice. In the experiments of La Mer, Campbell and Sherman upon the vitamin as it exists in filtered tomato juice, the velocity of the heat destruction decreased more rapidly than it would if the reaction followed either the unimolecular reaction law or the square root rule of Schutz. Empirically the percentage destroyed was found to vary as the fourth root of the time of heating. The temperature coefficient of the rate of destruction is lower than those of ordinary chemical reactions, for while a rise of 10° C. usually doubles the rate of an ordinary reaction, that of the destruction of vitamin B in cabbage was multiplied only by about 1.3 (Delf) and in tomato juice only by about 1.2 (La Mer, Campbell and Sherman). The low temperature coefficients and the difference in rate of destruction within and without the plant cells suggest that the reaction involved in the heat destruction of the vitamin is of the heterogeneous type.

The studies of the heat destruction with reference to time and temperature warrant the general view that for short periods of time (one hour or less) the reaction runs rapidly and therefore in such cases the length of time of heating is likely to be a more serious factor than the temperature, while for longer periods (four hours or more) the effect of temperature assumes relatively greater prominence in comparison to time than it does in shorter periods. Thus in the experiments with tomato juice, the results of which are summarized in Fig. 7 above, the destruction of vitamin at the end of 30 minutes heating at 80° was about 27 per cent and at 100° about 30 per cent; whereas at the end of four hours heating the difference was much greater, namely 53

per cent at 80° and 68 per cent at 100°. In practice this would mean that when a desired effect in cooking or canning can be accomplished much more quickly by using a higher temperature it will probably be advisable to do so; but that when food is to be kept for a considerable time it becomes important from the vitamin standpoint that the lowest practicable temperature be maintained. Hence the better preservation of "freshness" and antiscorbutic value in cold storage than in storage at ordinary temperature, and the serious loss of this property when food is kept hot for a long time between cooking and serving.

The low temperature coefficient of the heat destruction of vitamin C, its solubility in alcohol, and its diffusibility, all count strongly against the view that the vitamin may be an enzyme-like substance. The typical enzymes are much less soluble in alcohol and dialyze less readily than appears to be the case with the vitamin, and their heat destruction is enormously accelerated by temperature, while that of the vitamin is accelerated to a surprisingly low degree. It is true that we frequently meet definitions of enzymes couched in such broad terms as would probably cover vitamins as well, but the difference just mentioned is such as to make it appear fairly certain that typical vitamins are substances of considerably simpler constitution than typical enzymes.

Vitamin C is more stable in an acid than in a neutral medium, and still less stable when the medium is alkaline. In the above mentioned experiments with tomato juice the neutralization of less than half the natural acidity of the juice increased the destruction of vitamin C which occurred during one hour's boiling from 50 per cent to 58 per cent. Even in storage at low temperatures the loss of antiscorbutic value is much more rapid in a food which has been rendered slightly alkaline than in one which is distinctly acid.

Drying and aging often result in considerable losses of vitamin C but these losses are probably determined by the factors time, temperature, hydrogen ion concentration and exposure to oxidation. By sufficient attention to these factors, foods may be dried or canned and preserved for months certainly, and doubtless for years with little loss of their antiscorbutic vitamin. While it is difficult to dry foods thoroughly without heating, it is altogether probable that the subsequent preservation of the

vitamin C not destroyed during the drying process is favored by the absence of water.

The antiscorbutic vitamin is apparently not appreciably sensitive to ordinary light (La Mer). To ultraviolet light also it appears to be relatively stable, resembling vitamin B in this respect, while vitamin A is relatively sensitive (Zilva).

Chapter IV.

The Fat-Soluble Vitamin—Vitamin A.

While the conception of the presence in certain food materials of the water-soluble vitamins B and C developed primarily from observations upon disease, the conception of the fat-soluble vitamin arose, as has been noted, from the failure to secure normal growth in experimental animals for a long period of time on purified food materials furnishing adequate proteins, fats, carbohydrates and salts. The plan of studying the nutritional needs of animals through the feeding of mixtures of purified foodstuffs instead of natural foods had early impressed itself as the logical method of procedure but one beset with difficulties. Voit (1881) in his treatise on nutrition in Hermann's *Handbuch der Physiologie* wrote: "Unquestionably it would be best for the purpose if one could feed only pure chemical compounds (the pure foodstuffs), for example, pure protein, fat, sugar, starch, ash constituents, or mixtures of the same. However, inasmuch as men and animals only rarely tolerate continuously such tasteless mixtures, it is necessary in most cases to choose foods as they are provided by nature." Several workers contributed links to the chain of evidence of the existence in natural food materials of unknown growth-promoting substances as noted in the introductory chapter. Lunin's experiments conducted primarily to determine the significance of certain inorganic substances in nutrition led to the suggestion that milk must contain unknown substances indispensable in nutrition. Stepp (1909) published the first of a series of experiments concerned with the indispensability of lipoids for normal nutrition. His procedure was to feed mice with milk-bread subjected to prolonged extraction with alcohol and ether for the purpose of removing the lipoids. In commenting on the use of this food instead of an artificial, synthetic, lipoid-free mixture he stated that, as we had no exact knowledge of the materials necessary for life, nutritive failure on a "synthetic" ration lacking in lipoids might be attrib-

uted not to lack of lipoids but of other unknown substances. Stepp found that mice could not live on the extracted bread but that if the total extract were fed with the bread normal growth ensued. In the light of our present knowledge of vitamins, the results obtained by Stepp in his effort to identify the indispensable substances removed from the food materials by extraction with alcohol and ether, as reported in this and later studies, can readily be interpreted from the known properties of the water-soluble and fat-soluble vitamins, both soluble in alcohol and the latter in ether and both consequently removed from the materials used in his experiments. Unable to identify the removed indispensable component as any of the known lipoids, Stepp (1911, p. 150) called attention to the property of lipoids of influencing the solubility of other materials to such an extent that they also would be removed during the extraction. "So wäre es nicht undenkbar dass gemeinschaftlich mit den Lipoiden irgendwelche unbekannte lebenswichtige Stoffe in Lösung gehen und dass so die Lipoide gewissermassen zu Trägern für diese Stoffe würden, dass mit anderen Worten bei der Entfernung von Lipoiden die unbekannten Körper mit enfernt und bei Zusatz von Lipoiden mit diesen zugesetzt werden. Ein Hinweis auf eine derartige Möglichkeit erscheint notwendig, solange es nicht gelingt, die Versuche mit chemisch reinen Körpern durchzuführen."

The work of Osborne and Mendel during this same period was directed largely to the question as to whether normal nutrition could be secured upon a dietary containing a single individual protein instead of mixtures of proteins such as normal dietaries comprise. They soon came to emphasize also the importance of factors other than protein and discovered that mixtures of purified foodstuffs which were capable of maintaining for some time the life of full-grown rats were unable to support the growth processes in young animals. Favorable experiences with milk and milk powder led to many unsuccessful attempts to modify the inorganic and non-protein ingredients of the diet of purified foodstuffs and finally to the use of the so-called protein-free milk which was prepared by removing as far as possible by precipitation and coagulation the proteins from the fat-free milk, evaporating the filtrate and grinding the residue to powder. This material, which was added to the artificial dietaries in liberal amounts (about 28 per cent of the total food mixture), was found

to promote rapid growth in rats and was at first thought to solve the problem of artificial dietaries.

Shortly after the publication of Osborne and Mendel's work involving the use of protein-free milk, Hopkins (1912) published the results of his experiments conducted in 1906 and 1907 on the remarkable effect produced in the growth of rats on a diet of purified food substances by the addition of small amounts of milk or the alcohol-soluble portion thereof. That his observations had at an even earlier date suggested the idea that natural food materials contain other essential ingredients than those hitherto recognized is shown by his statement in 1906 noted on page 8. The delay in publishing the remarkable results which he had obtained has been explained in a recent note regarding his early experiments in which Hopkins (1921) states, "The experiments which I described in 1912 followed upon a long experience of the effects of adding tissue extracts and especially fractionated yeast preparations to purified diets. Looking back to one's experience in these years during which startling successes were mingled with puzzling failures—failures which led to delay in publication—I realize that the absence of all knowledge concerning the factor associated with fats was the cause of any experimental contradictions. Had I possessed the scientific vision which afterwards led McCollum and Osborne and Mendel to recognize the existence of this, I should have reached full conviction as to the reality of my results long before I did. In the synthetic diets employed by me the proteins and the carbohydrates were purified to the utmost, but I used little or no discrimination with regard to the fats."

The work of McCollum and of Osborne and Mendel to which Hopkins referred appeared almost simultaneously in 1913. McCollum and Davis, in the course of a study of the influence of the composition and quantity of the inorganic content of the ration on growth in the rat, employed rations of purified casein, carbohydrates and various salt mixtures and the same rations in which part of the carbohydrate was replaced by lard. It was found that with certain proportions of the various ingredients normal growth of the animals for periods varying from 70 to 120 days often resulted, while beyond that time little or no increase in body weight could be induced, although the animals remained in apparently good nutritive condition for some time. After

numerous attempts to promote further growth by adjustments between the various ingredients of the food mixture, it was found that resumption of growth occurred quite promptly after the introduction into the diet of the ether extract of egg or butter. Negative results were obtained with lard and olive oil, thus showing that the suspension of growth was not due merely to the absence of fat from the diet. The conclusions drawn at this time, as taken from the authors' discussion, are as follows: "Whether the resumption of growth is the result of supplying in the ether extract of egg or of butter some indispensable organic complex of the chemical nature of the lipins, or is the result of a stimulating action of some substance accompanying the lipins can not be decided from the data available. . . . Our observation that ether extracts from certain sources improve the condition of animals on such rations strongly supports the belief that there are certain accessory articles in certain foodstuffs which are essential for normal growth for extended periods." Similar conclusions published by Osborne and Mendel (1913) during the same summer were arrived at from an attempt to explain the great superiority of diets employing milk over purely artificial food mixtures or even mixtures containing protein-free milk. A mixture of milk powder 60, starch 12, and lard 28 per cent proved to be adequate for growth and maintenance while "protein-free milk" food consisting of protein in the form of edestin or casein 18, starch 26, lard 28, and "protein-free milk" 28 per cent failed to support growth for more than about 100 days. The essential difference evidently lay in the absence from the protein-free milk foods of those components of milk which are separated in the process of centrifugation. This led to the substitution for part of the lard in the protein-free milk food of a corresponding quantity of butter fat, a change which brought about prompt recovery and rapid growth of the experimental animals and which justified the authors in concluding "It would seem, therefore, as if a substance exerting a marked influence upon growth were present in butter, and that this was largely, if not wholly, removed in the preparation of our natural 'protein-free milk.' Whether or not the latter is wholly deficient in this substance can not be determined as yet from any data we possess. It is true that young rats are able to make very considerable growth when fed on the natural 'protein-free milk' diet, but possibly

Fig. 8.—Photograph of rat showing effects of diet lacking vitamin A. In this case the effects are apparent in the eye, ear, nose, feet and coat. See accompanying text and discussion later in this chapter. (Courtesy of Doctors Osborne and Mendel.)

this is accomplished at the expense of some reserve substance stored in the cells of the young animal." The ability of the animal body to store the fat-soluble vitamin, thus early suggested by Osborne and Mendel, has proven to be a matter of much importance.

Further study by Osborne and Mendel of the influence of butter fat on growth, reported later in the same year (1913a) showed that the active ingredient was contained in the clear fat fraction of butter, was essentially free from nitrogen and phosphorus and devoid of any ash-yielding material, and evidently not destroyed when steam was passed through the melted butter fat. In discussing the nutritive superiority of butter fat over lard, the authors note at this time that failure at certain periods of the year, particularly in the summer months, to secure satisfactory growth on dietaries which had proved adequate at other times could be averted by the addition of butter fat to the usual protein-free milk food mixtures and further that a "type of nutritive deficiency exemplified in a form of infectious eye disease prevalent in animals inappropriately fed is speedily alleviated by the introduction of butter fat into the experimental ration." This characteristic eye condition (Fig. 8) will be discussed more fully later. Further work of Osborne and Mendel on this fat-soluble, growth-promoting substance (vitamin A) showed it to be present in cod liver oil, but absent in freshly pressed almond oil (1914), and to be present in small amounts in beef fat (1915a). When butter fat and beef fat were subjected to fractional crystallization from alcohol, the active substance was found to be concentrated in the mother liquor or oil fractions rather than in the fractions containing fats with high melting points. Heating butter with live steam two and one half hours did not destroy its properties nor did storage in the light or dark under ordinary conditions (1915). The butter oil in which the growth-promoting substance is more concentrated than in the original fat showed gradual deterioration, losing most of its potency in a year.

Meantime McCollum and Davis (1914) had shown that the growth-promoting substance in butter was sufficiently stable to withstand the saponification of the butter in alcoholic potassium hydroxide and that it could be shaken out from the saponified material by olive oil. In an attempt to determine whether the

substance was carried by vegetable as well as animal fats (1915) rats which had been brought to a state of emaciation on a fat-free diet of casein, milk sugar, dextrin, agar, and salts were brought back to a normal condition by the substitution of corn meal for 50 per cent of the fat-free diet. Similar results were obtained with wheat embryo (1915a), but less favorable with whole wheat flour, rye flour, and oats. Reproduction was not secured in any of these experiments. In the following year McCollum, Simmonds, and Pitz (1916b) published results which indicated that oils such as maize, cottonseed, linseed, olive, sunflower seed, and soy bean oils in amounts up to from 10 to 20 per cent of the ration do not furnish fat-soluble vitamin in appreciable amounts. On the other hand, alfalfa and cabbage leaves were found to be an excellent source of the vitamin. The cereal grains, while containing a small amount, were markedly inferior to the alfalfa leaves. The authors conclude at this time that "the superiority of the forage portion of the plant over the seed with respect to its content of the fat-soluble A is of considerable interest when viewed in the light of the dietary habits of lower animals. Those which consume the forage rations grow successfully from generation to generation on a strictly vegetarian diet, while the seed-eating animals, so far as we have been able to learn, normally vary their diet to a considerable degree by the addition of green leaves, worms, insects, etc." They further conclude from the observation that the ether-extracted residue of corn meal is more effective in causing growth than is corn oil, that "ether extraction of plant tissue does not remove the substance essential for growth which is contained in butter fat. The obvious working hypothesis must for the present assume that the fat-soluble A is in chemical union in the plant tissues, and in a complex which is not soluble in fat or in ether. In digestion and absorption it is set free and being readily soluble in fats, thereafter accompanies the fats in the animal body."

That neither vitamin A nor B could be synthesized by the animal was indicated by the failure of rats on diets deficient in one or the other of these vitamins successfully to suckle their young except through a sacrifice of their own tissue and then to only a limited extent (McCollum, Simmonds and Pitz 1916c).

In September, 1917, Halliburton and Drummond reported in detail an extended research dealing with the nutritive values of

margarines and butter substitutes with reference to the fat-soluble vitamin, the results of which led them to conclude that the only butter substitutes of the many examined which can adequately replace butter are the margarines made from the so-called "oleo-oil" from beef fat.

The foregoing sketch attempts to review the literature of the fat-soluble vitamin in chronological sequence to the end of 1917. Later investigations have proceeded along such divergent lines that it seems best to consider them under separate phases rather than purely chronologically.

Physical and Chemical Properties of Vitamin A.

Lack of knowledge of the properties of the fat-soluble vitamin, particularly as regards its solubility, stability and storage, has led to much confusion concerning its quantitative distribution in food materials. Consequently the earlier literature should be studied rather critically from the standpoint of present knowledge concerning its behavior under various experimental conditions.

Solubility and extraction. As noted above, an apparent difference between the solubility of vitamin A when associated with animal fats and when in plant tissues was early reported. In association with animal fat it was found to be readily soluble in ether and other fat solvents and slightly soluble in alcohol. (Osborne and Mendel, 1915a; Drummond, 1919a). It was at one time stated by McCollum, Simmonds and Steenbock (1917) that shaking melted butter fat with repeated change of water removed the active substance from the fat. Attempts by Drummond to repeat this method of extraction failed and later one of the authors responsible for the original statement modified it by reporting (Steenbock, 1918) that neither the washed butter fat nor the aqueous extract contained the vitamin. In the light of recent experiments concerning the stability of vitamin A, it is readily seen that the process of repeated shaking of the butter fat with hot water in the presence of air might readily have destroyed the vitamin. Other attempts at extracting the vitamin from fats were chiefly concerned with unsuccessful efforts to discover the chemical nature of the vitamin and will be considered later.

Osborne and Mendel (1919, 1919d) reported that they had been able to obtain potent preparations of fat-soluble vitamin from plant tissues such as spinach leaves and young clover by drying the material in a current of air at about 60° C., extracting with U. S. P. ether and evaporating the extract on starch. These preparations fed in daily quantities equivalent to from 1 to 2 grams of the dried plant promoted recovery and renewal of growth in rats on diets deficient in fat-soluble vitamin. These results seem to be at variance with those reported earlier by McCollum.

Steenbock and Boutwell (1920b) attempted to concentrate the fat-soluble vitamin from carrots, alfalfa, and yellow corn by fat solvents with the following results:

The fat-soluble vitamin in carrots was not removed to any extent by saturation with corn oil and extraction with ether, and apparently not at all by similar saturation with lard. The vitamin was slightly soluble in ether, somewhat soluble in chloroform and carbon disulphide, and quite soluble in alcohol and benzene, although no one of the extracts was sufficiently concentrated for practical purposes. With alfalfa more satisfactory results were obtained with ether, benzene, and alcohol, sufficient fat-soluble vitamin being obtained in the ether extract to permit satisfactory growth when fed to rats at a level equivalent to 20 per cent of alfalfa in the food mixture. These authors also employed with the alcohol extract from alfalfa meal the method commonly used for the separation of carotinoids. The extract was saponified with alcoholic potassium hydroxide and the mixture extracted repeatedly with ether until the yellow pigments were completely removed. The ether extracts, after washing with water to remove alkali and salts, were evaporated on a water bath to a small volume. This extract proved to be rich in fat-soluble vitamin. A further concentration of the vitamin was brought about by taking up a similar extract in a mixture of alcohol and petroleum ether and subjecting it to fractional extraction. The petroleum-ether-soluble carotin fraction was found to contain an abundance of the vitamin, while the alcohol-soluble xanthophyll fraction contained little or none of it.

About the same time Zilva (1920) reported experiments in which fractions active in vitamin A were obtained from fresh vegetables (carrots and cabbage) by extraction with absolute

alcohol and subsequently by ether. Absolute alcohol extracted in the form of a sirup about 7.5 per cent of dry matter from fresh carrots or about one half of the total solids. This sirupy extract given daily in amounts equivalent to from 10 to 12 grams of fresh carrots provided sufficient vitamin A to promote normal growth in rats subsisting on a diet otherwise lacking this vitamin. On extracting with ether the concentrated extract obtained by evaporation of the alcohol, an oily residue remained which, in amounts equivalent to 25 grams of fresh carrots, promoted recovery and renewed growth in rats declining on a basal diet lacking in vitamin A.

Thus present evidence indicates that vitamin A may be removed from either animal or vegetable material by extraction with alcohol followed by ether. This has led to the custom of subjecting materials to be used in a basal ration free from vitamin A to prolonged extraction with alcohol and ether. Drummond and Coward (1920) have emphasized the necessity of such extraction, stating that in their opinion "it is frequently the composition of the basal dietary which is responsible for the many misleading and contradictory statements which tend to confuse the literature on the vitamins at the present time."

Stability to heat and oxidation. Similar confusion has existed in regard to the stability of vitamin A. In their early work McCollum and Davis reported that the factor was present in the ether extract of boiled eggs and Osborne and Mendel (1915a) found that butter fat treated with steam for two and a half hours did not appear to have lost its growth-promoting properties. The fat-soluble vitamin was generally considered a thermostable substance and in experimental work on its occurrence in foods but little thought was given to the possibility that the manipulation involved, such as shaking of the butter fat with water, or subjecting materials to prolonged drying with artificial heat, might destroy their activity. In 1918, however, Steenbock, Boutwell and Kent, in the first of a series of studies on the fat-soluble vitamin, reported that vitamin A was comparatively labile to heat. Melted butter fat shaken with water for 12 hours became inactive as did butter aerated for 12 hours at 100° C. The possibility that the destruction was due to oxidation was apparently disproved by similar results obtained when butter was shaken with carbonated water. Butter heated in jars at 100°

in a Freas oven also lost its activity as did butter kept unsalted under poor storage conditions for three weeks. The authors conclude that "the failure of other investigators to note this destructive action was undoubtedly due to the high initial content of fat-soluble vitamin in the material studied. The destructive process is evidently a reaction of low velocity. With large amounts of vitamin present and with heat treatment for a limited period of time sufficient amounts of the vitamin remained to satisfy all the requirements for normal growth in the experimental animals."

Shortly afterward, Drummond (1919a) in an investigation of the effects of hydrogenation on the vitamin content of oils, found in experiments with whale oil that the hardening process involving exposure to hydrogen gas at 250° C. for four hours or more destroyed the vitamin as did heating the oil to 100° or more for four hours. This investigation yielded results which were thought to demonstrate beyond any doubt that fat-soluble A in the form in which it occurs in natural animal fats is much less stable to high temperatures than had previously been assumed. "Such an observation necessitated a complete revision of all the previous experiments to isolate and identify the factor in many of which high temperatures had been employed." In a series of experiments reported at this time on the effect of heat on the vitamin present in certain oils, the results obtained supported the conclusions of Steenbock that the vitamin was comparatively easily destroyed by heat. The possibility that the destruction was a result of oxidation or hydrolysis was believed to be excluded by determinations of the acid value and iodine number of the active and inactive oils. No relationship was found to exist between these constants and the state of destruction. The conclusion drawn at this time was that "the chief agent in the inactivation of fat-soluble A is temperature. It must, however, be borne in mind that all these speculations refer solely to the factor as present in secondary sources such as the animal oils. It is quite possible that the factor occurs in a different form in its primary environment in the plant tissues."

That vitamin A as it exists in animal fats is stable to heat under certain conditions was shown by Osborne and Mendel (1920c) who published a confirmation of their earlier work indicating that the vitamin present in butter is notably stable at

relatively high temperatures. Butter fat treated with steam and also heated at 96° C. for 15 hours retained its growth-promoting activity. This explanation of these conflicting results came in papers published simultaneously by Hopkins (1920a), by Drummond and Coward (1920b), and by Zilva (1920a), all of which present evidence that the destruction of vitamin A takes place rapidly at high temperatures when oxygen or an oxidizing agent is present, but not otherwise.

The method adopted by Hopkins was to feed young rats of the same age, size, and sex, a ration free from vitamin A to which was added 15 per cent of filtered butter. The butter fed to half of the animals in each group had been heated in the autoclave for different lengths of time, while that fed to the other half had undergone heating at the same temperature but with aeration. In all of these experiments growth was normal on the unaerated fat; but on aerated fat growth ceased and in 60 per cent of the cases xerophthalmia developed. As judged from the growth curves, 4 hours' exposure to a temperature of 120° in the absence of air did not appreciably affect the vitamin content of butter, at least when fed at 15 per cent of the food intake; 12 hours' exposure under the same conditions involved slight destruction, aeration at 120° for 4 hours destroyed the greater part, and for 12 hours practically all of the vitamin; aeration at 80° brought about quite rapid destruction, and exposure to air at ordinary temperatures for a week almost complete destruction. Of significance in this report was the fact that identical figures were obtained for the iodine value of the fatty acids of the butter before and after heating for 4 hours in a stream of air at 120°.

The plan adopted by Drummond and Coward (1920b) differed from that of Hopkins in that the butter fat was fed in small amounts (0.2 gram) as a supplement to the daily vitamin A-free basal ration to rats whose growth had been completely inhibited by this ration. The temperatures and time of aeration were: Exposure to a current of live steam for 6 hours, and heating at 96° for 15 hours, at 50° for 6 hours, and at 37° for 3 weeks with and without exposure to air. The results of these studies also indicate that destruction of vitamin A takes place rapidly at high temperatures and to a considerable extent at temperatures as low as 37° in the presence but not in the absence of air.

The confirmatory work of Zilva (1920a) published at the

same time resulted from an earlier observation that butter fat is inactivated by exposure to ultraviolet rays (Zilva, 1919). In a further study to determine whether this was due to the action of the rays or to ozone produced by the mercury quartz lamp, codliver oil was exposed in thin layers to the action of ultraviolet light in an atmosphere of carbon dioxide gas for 16 hours, at the end of which time it was found to have lost none of its activity. On exposure for from 6 to 10 hours to a current of ozone in a bottle impervious to light it became so inactivated that large doses failed to promote growth in rats on a diet otherwise deficient in vitamin A, showing that ozone inactivates the vitamin, a finding in complete agreement with the observations noted above, the action of ozone being more drastic and therefore more rapid than aeration.

Accepting these findings as conclusive, the discrepancies in earlier conclusions concerning the stability of vitamin A are readily understood. In cases where stability was reported, as in the exposure of butter fat to live steam, the conditions of the experiment gave an air-free medium, while in such experiments as the removal of vitamin A from butter fat by repeated shaking with hot water, in the presence of air, the destructive action was probably one of oxidation. It should be noted, however, that in all the experimental work reviewed above, animal fats, sometimes referred to as the secondary source of vitamin A, have been used. Up to the time of writing no experiments so crucial as the above have been reported concerning the stability of vitamin A in plant materials.

Steenbock and Boutwell (1920a) determined the stability of the fat-soluble vitamin in plant materials by means of feeding experiments in which the vitamin-containing material was dried at room temperature, soaked in water, autoclaved for 3 hours at approximately 15 pounds pressure, and again air-dried before being incorporated into the ration. This treatment appeared to cause no noticeable destruction of the fat-soluble vitamin of yellow maize, chard, carrots, sweet potatoes, and Hubbard squash. The results obtained with dried alfalfa were not so favorable, although this was fed at a level at least twice as high as necessary for normal growth. The rats on this diet after a normal growth for 3 or 4 weeks declined and died, some showing signs of xerophthalmia. While at this time the effect of heat

rather than possible destruction by oxidation through exposure to air in the drying process was the one chiefly considered, the authors call attention to the fact that the alfalfa meal used was a commercial product, the history of which was not known, and that possibly while the fat-soluble vitamin was not destroyed by the heat treatment itself, it was made more susceptible to destruction—possibly by liberation from combinations—by the agents operative in the aging process.

That vitamin A as it exists in plant tissues is not in a form to be as easily acted upon by oxidation as it is in animal fats is indicated from the ability to prepare highly potent materials by extraction of air-dried vegetables as illustrated by recently reported work of Osborne and Mendel (1920c). Alfalfa, clover, timothy, and spinach freshly dried at 60° C. in amounts of 0.1 gram of the dried substance furnished as much vitamin A as did 0.1 gram of butter fat, while with tomatoes more rapid growth resulted from the addition of 0.1 gram of the dried material than of the same amount of butter. In this case, however, the rapid growth is attributed partly to the richness of the tomato in vitamins B and C.

The problem of destruction of vitamin A in vegetables is one deserving of careful study inasmuch as green vegetables are relied upon to a considerable extent in some regions to make good (in part) the deficiency in the milk supply. Drying of green vegetables is becoming a matter of industrial importance and in the process of drying, means should be taken to render any deterioration of vitamin values as slight as possible.

Indications as to Chemical Nature or Relationships.

McCollum and Davis (1914) saponified butter fat in absence of water and were able to extract the fat-soluble vitamin from the soap by shaking with olive oil which latter then showed the growth-promoting property not shown by the olive oil originally. Drummond (1919) has described similar experiments in somewhat greater detail. Fats (butter and whale oil) were saponified by Henriques' method at room temperature, the solvents removed in vacuo at 35°, the soaps dissolved in distilled water at 35° and the unsaponifiable matter removed by extraction with ether. The fatty acids were liberated by slight excess of mineral acid

and removed by ether extraction. Both fractions after washing with water and evaporating under reduced pressure at 37° showed no activity as a source of vitamin A.

These results were thought to indicate that the fat-soluble vitamin is not a fatty acid, but that, contrary to the above results of McCollum and Davis, it does not withstand saponification at room temperature. Recently Coward and Drummond (1921) have reported, however, that if oxidation is prevented it is possible to obtain vitamin A in a highly concentrated form by cold or hot saponification of animal oils or plant tissues. Drummond (1919) also found that the fat-soluble vitamin could not be replaced in the diet by a number of the commoner fatty acids, glycerol, cholesterol, lecithin, sphingosin, phrenosin, kephalin and the lipochrome, carotin, and so cannot be identical with any of these substances. He also showed that it behaved differently from the nitrogenous bases and from vitamin B. The suggestion advanced by Drummond at that time was that "the accessory growth-promoting factor provisionally termed 'fat-soluble A' is not a clearly defined chemical substance but rather that it is a labile substance perhaps possessing characteristics resembling those of an enzyme. It is surely legitimate to conceive the animal organism being as dependent upon the plant kingdom for a supply of an essential complex of that nature as it is for the supply of many indispensable substances of more clearly defined constitution."

As the list of materials containing fat-soluble vitamin increased a certain analogy was noted and first pointed out by Steenbock (1919) between the simultaneous presence of the carotinoid pigment and fat-soluble vitamin in certain foods and their absence in others. Palmer had earlier shown (1915, 1916) that the plant carotinoids are the source of the so-called lipochromes of the higher animals by direct transfer of the pigments of the diet. Experimental work reported by Steenbock and Boutwell (1920) shortly after the preliminary statement of the hypothesis of a relationship between the fat-soluble vitamin and the yellow pigment carotin, showed that yellow corn furnished enough of the fat-soluble vitamin to allow growth at the normal rate to take place in the rat and to make possible reproduction but not rearing of the young; while the feeding of white corn under similar conditions resulted in nutritional failure. Red

corn with a white endosperm gave the same results as white corn, while that with a yellow endosperm gave results approximately the same as those from yellow corn. Later papers by the same author and his co-workers have furnished many data in harmony with the theory of a relationship between vitamin A and lipochrome pigment. As noted above, fractionation of the unsaponifiable matter from alfalfa hay brings down the fat-soluble vitamin in the carotin-rich fraction. One report of the work of these authors (Bulletin No. 323 of the Wisconsin Agricultural Experiment Station, 1920) contains the statement that preparations have been made in crystal form which have brought about immediate recovery and rapid resumption of growth when fed to rats in a nutritive decline.

The above theory at first received the support of Rosenheim and Drummond (1920). The failure of lipochromes to serve as sources of fat-soluble A as previously noted by Drummond (1919a) was considered to justify the conclusion that the lipochrome pigments and vitamin A are not identical but not to refute the theory of a close relationship between them. Later Drummond and Coward (1920) subjected this theory to the test of comparing a large number of animal and vegetable oils as to their relative richness in vitamin A and in lipochrome pigments. The lack of relationship between the pigmentation of the 24 fats examined and their richness in vitamin A led them to conclude that "unless we assume the existence of a leuco-form it does not appear probable that the fat-soluble vitamin is a member of the lipochrome class of pigments. The frequent association of the growth factor with pigments of that type must therefore be regarded as accidental." In this they agree with the conclusions of Palmer and Kempster (1919, 1919a) who reported success in raising a large number of White Leghorn fowls from hatching to maturity on rations which contained at the most mere traces of carotinoids. Hens on this ration laid eggs which were practically free from pigment and from which normal chickens were hatched. Furthermore Stephenson (1920) found that a crude (alcohol-light petroleum) extract of dried carrot when added to a fat lacking in vitamin A conferred upon it growth-promoting properties and protected rats from xerophthalmia while pure carotin extracted from carrots was without effect. Moreover, butter from which the coloring matter had been com-

pletely removed by filtration through charcoal did not lose its growth-promoting properties.

Quite recently the literature on this subject has been subjected to an extensive and critical review by Palmer, Kennedy and Kempster (1921) who have also presented perhaps the most cogent evidence yet advanced in opposition to the idea of identity or relationship between vitamin A and carotinoids. They not only report a complete absence of carotinoid pigments in the albino rat, the experimental animal used in most of the biological studies on vitamin A, but were able to report growth and reproduction of rats with ewe milk fat containing only 0.00014 per cent carotin as the sole source of vitamin A, the ration showing the best results containing only 0.0000126 per cent carotin. Growth and reproduction were also obtained with rats using carotinoid-free egg yolk as the sole source of vitamin A. Quantitative comparisons were made of the carotin content and vitamin A efficiency of various rations as reported in the literature. The results of this comparison indicated that carotin and vitamin A are not quantitatively associated in the plant tissues in which both are presumably synthesized. The existence of a leuco-form of the vitamin as suggested by Steenbock (1919) to cover the exceptions to the association of pigment and vitamin is thought by Palmer to be unlikely in view of the fact that the only leuco-forms of carotinoids so far produced are oxidation products and that oxidation destroys the efficiency of the fat-soluble vitamin.

Even more recently, however, Steenbock and his associates have published further evidence of a tendency toward greater richness in vitamin A in association with higher carotin or lipochrome pigmentation. Thus Steenbock, Sell and Buell (1921) point out that "with the diversification of metabolic processes which obtain in the plant and animal kingdom, it was to be expected that sooner or later the fat-soluble vitamin would be found to be present in a menstruum entirely free from pigments of the carotinoid type. To run across such an instance, appears to have been the good fortune of Palmer and Kempster (1919) who demonstrated that pork liver, rich in the vitamin, contained no pigments of the aforementioned character." . . . "Nevertheless, as far as studies in this domain have been pursued, both in regard to distribution of vitamin and pigment and in regard to

their physical and chemical properties, there is left no doubt but that chemically and physiologically they are related." These authors then give experimental results of their own showing high vitamin A value with very low pigmentation in codliver oil, and further that "The fat-soluble vitamin content of butter fat does not run closely parallel to the yellow pigment; yet in general, due to determination by their content in the feed, butters highly pigmented are rich in the vitamin; butters low in pigment should be looked upon with suspicion. In beef fats the relations are somewhat similar; those most pigmented are also generally richest in their fat-soluble vitamin content." In this same paper experiments, extending the previous work of McCollum, are described which lead to the conclusion that fat-soluble vitamin withstands severe methods of saponification.

In the next paper of the series, Steenbock, Sell and Boutwell (1921) record feeding experiments with six varieties of peas showing that "those of a green color, also carrying considerable yellow pigment, were far richer in their fat-soluble vitamin content than yellow peas which contained much less yellow pigment."

To the writers of this monograph it would seem from the evidence available up to the middle of 1921, that while there is no absolute parallelism between vitamin A and pigmentation, yet the instances of apparent correlation in natural products are too frequent to be dismissed as merely accidental and are rather to be regarded as affording a useful though not conclusive indication of some chemical or biological relationship which still remains to be worked out.

Recently Coward and Drummond (1921) have attempted to trace the origin of vitamin A in plants by testing seeds, germinated seeds, etiolated seedlings, green seedlings and the older green plants for the relative content of vitamin A by the method which they have described. Preliminary tests indicated the absence of vitamin A in the seeds of turnip, cabbage, white maize, and sycamore and its presence to a greater or less extent in peas and yellow maize. Cress seeds and shoots were refused by the rats. No increase in the amount of vitamin A in the germinated seeds could be detected. The results with etiolated seedlings administered in amounts of about 0.7 gram per day were somewhat inconclusive, but later more carefully controlled experiments indicated that the content of vitamin A is not appre-

ciably greater in the etiolated seedlings than in the original seeds. Green shoots of turnip, maize, and peas (soil and sand grown) were decidedly richer in vitamin A than the original seeds or etiolated seedlings. A more detailed study with the sunflower confirmed in general the preliminary results. The dry seeds and etiolated shoots were relatively inactive, while the green shoots were very active as a source of vitamin A, thus suggesting that the formation of large amounts of vitamin A in green leaves requires the influence of light.

Evidence is also furnished in this contribution that vitamin A can be produced by the green plant from inorganic sources as shown by its high content in the green shoots of Tradescantia (Wandering Jew) grown in water; that green cabbage is much richer in vitamin A than white cabbage, that mushrooms contain only a small amount of vitamin A, and that common green seaweeds (Ulva and Cladophora) are as potent in vitamin A as the green land plants such as cabbage, while red seaweed (Polysiphonia) and Carrageen moss (Chondrus crispus) have no appreciable amounts of vitamin A. All these observations point to the greater activity with respect to vitamin A of chlorophyll-containing plants.

Occurrence and Estimation of Vitamin A in Animal and Vegetable Tissues and Products.

As yet our knowledge of the distribution of this substance is dependent upon the results of feeding experiments which apparently do not warrant statements of such a quantitative character as are now possible regarding relative amounts of antiscorbutic vitamin, but do serve to establish the fact that some foods are rich in vitamin A, others contain little if any of it, and still others occupy an intermediate position in this regard.

The starting point of our knowledge on this subject was the discovery by McCollum and Davis (1913) that rats could not be nourished satisfactorily upon certain food mixtures of which lard was the sole fat; but the use of butter fat sufficed to make the food mixture adequate. Further experiments showed that, with the samples used, no more than 5 per cent of butter fat need be contained in the food mixture to make it adequate in this respect, whereas even 28 per cent of lard did not suffice. Evi-

dently, then, butter fat is rich in this nutritive essential (vitamin A) and lard either lacks it entirely or contains so little as to show no evidence of its presence in such experiments.

Through the work of Osborne and Mendel and of McCollum and his associates it was shown that egg fat, codliver oil, and the fat of pigs' kidney resembled butter in serving as efficient sources of vitamin A while cottonseed oil, olive oil, almond oil and other commercial vegetable fats seemed, like lard, to lack it. The skeletal muscles (ordinary meats) contain but very little vitamin A; heart muscle, somewhat more, but not so much as liver or

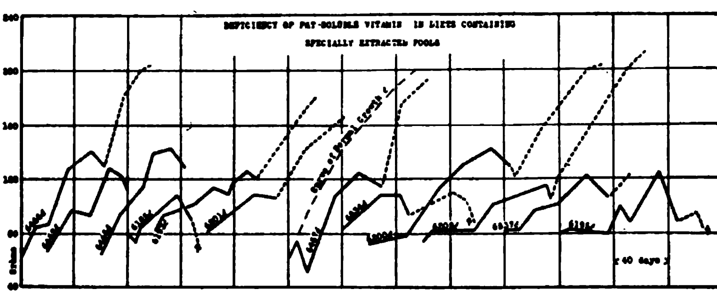


FIG. 9.—Weight curves of rats on diets lacking vitamin A from experiments by Osborne and Mendel (1921). The curves show that growth continued for differing lengths of time at different rates after the animals were placed upon food which had been carefully freed from vitamin A. The interrupted lines show periods of feeding with vitamin A after growth had stopped and animals had shown pathological effects from lack of this vitamin. In most but not all of these cases cures were effected and normal growth resumed. (By permission of the *Journal of Biological Chemistry.*)

kidney. The finding by Osborne and Mendel (1915a) that beef fat, while not so rich as butter, yet contains this vitamin in significant amounts, makes it seem all the more strange that lard (the corresponding body fat of the pig) should contain none of it, and recently Daniels and Loughlin (1920) and Drummond, Golding, Zilva and Coward (1920) have published new experimental evidence from which they conclude that lard and probably some other commercial forms of fat previously regarded as devoid of vitamin A are not wholly so, the vitamin value of lard (as probably also that of beef fat and of butter) depending largely upon the food of the animal.

That vitamin A may be stored to an important extent in the body is indicated not only by the results of feeding body fats and glandular organs as sole sources of this vitamin in the diet,

but also by the fact that when growing animals previously well fed are placed upon diets certainly deficient in vitamin A and often apparently devoid of it, they usually continue to grow for some time, often doubling their body weights before signs of nutritive deficiency appear. See the accompanying growth charts of rats fed by Osborne and Mendel (1921) upon diets made up of foods which had been especially extracted to free them from this vitamin (Fig. 9. When an animal placed upon a diet devoid of vitamin A ceases to grow at once, as has been reported in some cases, it may be because of insufficient food intake or because the previous food of the animal had not been such as to induce a storage of vitamin A in the body. As we now have much evidence that such storage is possible and that it depends largely upon the food consumed, we must expect that the vitamin A content of a given organ or tissue may vary considerably with different individuals of the same species. This means not only variability among the different specimens of a given type of food whose vitamin A content one may wish to test, but also the further complication of variable stores of the vitamin in the bodies of the test animals according to their age, previous feeding (Fig. 10), and possibly other conditions.

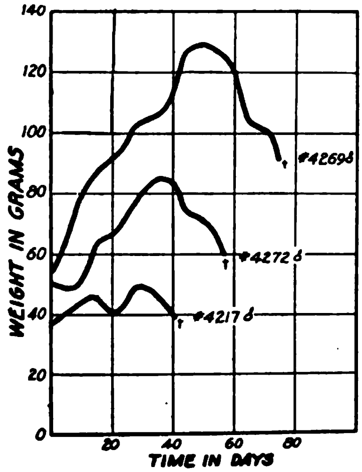


FIG. 10.—Showing the influence of previous feeding upon growth on diets lacking vitamin A. Rat 4217 had received a diet relatively low in vitamin A (⅚ whole wheat and ⅙ dried whole milk). Rat 4272 had received a diet richer in vitamin A (⅔ whole wheat and ⅓ dried whole milk). Rat 4269 had received a diet still richer in vitamin A (⅓ whole wheat and ⅔ dried whole milk). All were placed upon a diet devoid of vitamin A at the same age, 28 days. Growth and duration of life upon this diet was evidently determined by the different amounts of vitamin A stored in the bodies of the animals at the beginning of the experiment according to the richness in this vitamin of their previous diets. (Sherman and Boynton, unpublished data.)

Drummond (1919) has attempted to control some of the variables above mentioned by adopting the following technique in his studies of vitamin A: Young healthy rats selected from

home-bred stock and weighing about 50 grams each, are fed a ration consisting of purified casein 20, purified starch 50, salt mixture 5, yeast extract 5, butter fat 15, and filtered orange juice 5 parts. The animals which give evidence of a normal power of growth are removed from the complete ration when they have attained an average body weight of 70 to 80 grams and are given a similar ration in which the butter fat is substituted by an equivalent amount of hardened linseed oil. When it is definitely established that growth is inhibited by this deficiency in vitamin A, the linseed oil is wholly or partially replaced by the substance to be tested and the behavior of the animal closely watched for a period of from 5 to 6 weeks. Absence of vitamin A is indicated by failure to grow followed by a decline in health accompanied by the characteristic eye condition. Evidence of individual variability in reaction to this standardized technique is noted in the observation that while the experimental animals should quickly cease growing when fed the diet deficient in vitamin A "occasionally a very vigorous individual will continue to grow for some weeks after the deficiency has been introduced and such animals should not be employed in experiments of a quantitative nature."

Certain improvements in the technique of the feeding experiments were reported later by Drummond and Coward (1920). The basal ration as modified consisted of purified caseinogen 18, purified rice starch 52, refined vegetable oil (usually cottonseed) 15, yeast extract 5, orange juice 5, and salt mixture 5 parts. Rice starch was substituted for wheat starch as it was found to be almost devoid of vitamin A in the crude state and thus more readily purified than the wheat starch. On this ration it was reported that young rats of 50 to 70 grams weight should show only slight growth, becoming stationary after a week or two. All substances to be examined for vitamin A are tested on rats, the growth of which has been suspended for from 10 to 14 days and which do not weigh more than from 80 to 120 grams. The material is administered in definite weights to the animal before the day's basal ration is given. Drummond considers it important to observe the indicated limits of age and weight as he believes that the amount of vitamin A that must be supplied to a rat in order to restore growth which has been inhibited on a

deficient basal diet is inversely proportional to the age and weight of the animal. Recently Coward and Drummond (1921) have further altered the technique of preparing their basal ration, to the extent of purifying the caseinogen by exposing it in shallow layers to air at a temperature of 105° C. for at least 24 hours instead of extracting it with alcohol and ether as formerly. This simplified method is said to yield results quite as reliable as the older method, thus furnishing another indication of the ease with which vitamin A is now believed to be destroyed by oxidation.

Osborne and Mendel (1920c) have pointed out in attempting to correlate the varying results reported by different investigators as to the minimal amount of butter fat necessary for the maintenance of good growth in experimental rats, "it should be borne in mind that in contrast with what is true of animals having declined through complete deprivation of some requisite food factor the problem of dosage for vitamins may be quite different in the case of rats which are supplied with a minimum of the essential throughout the period of growth." In their experience much smaller amounts of the vitamin-containing foods are required when such foods are supplied from the start as a preventive measure than when given as a supplement after severe nutritive decline has set in. A typical basal ration employed by these authors in their study of vitamin A consists of meat residue 19.6, salt mixture 4, starch 52.4, and lard 24 per cent, with 0.4 gram daily of dry brewery yeast. The food to be tested for vitamin A is always given before the basal ration.

The basal ration employed by Steenbock and his associates in their extensive studies of vitamin A is essentially the same as that used by McCollum in his early studies. As reported by Steenbock, Boutwell and Kent (1918) and not altered substantially in later studies the ration consists of casein 18, agar 2, wheat embryo 8, salts 3.7, and dextrin 73.3 per cent. The casein was a commercial product purified by washing repeatedly with dilute acetic acid and the dextrin was partially dextrinized cornstarch prepared by heating cornstarch with dilute citric acid for 3 hours at 119° C. The wheat embryo was ether-extracted and in subsequent work an alcohol extract of this embryo was evaporated on some of the dextrin of the basal ration. Young rats 40 to 50 grams in weight are fed the basal ration alone for a

short period after which the substance to be tested for vitamin A is incorporated in the basal ration in place of equivalent amounts of dextrin. In describing this technique Steenbock and Gross (1919) state "We have repeatedly demonstrated that the basal constituents of our rations were too poor in vitamins to influence in any way the conclusion at which we have arrived, but on the other hand we make no claim that very small amounts of some vitamin may still not have been present and thus may have influenced in degree—though not in character—failure in maintenance, growth, reproduction and rearing of the young as indicated by the experimental rats."

Osborne and Mendel (1921) have recently called attention to the preliminary growth for a longer or shorter time on the deficient diet before nutritive decline sets in and venture the opinion that the problem of the removal of vitamin A from food products has not been satisfactorily solved. By subjecting the protein of the basal ration to three successive extractions with absolute alcohol under a reflux condenser for one hour they succeeded in reducing the period of undiminished growth but not in eliminating it altogether. They are of the opinion that removal of the fat-soluble vitamin from even purified protein and carbohydrates is accomplished with far greater difficulty than has been suspected hitherto. Another possible explanation advanced is that the amount of initial growth on diets deficient in vitamin A may be determined by the dosage of water-soluble vitamin which the animals can secure, a suggestion also made by Steenbock and Gross (1919, p. 50).

Hence it is plain that until experimental methods shall have been devised and generally adopted in which such sources of discrepancy are better safeguarded than in most of the work of the past, statements of relative amounts in different foods must probably be accepted with even more reserve in the case of vitamin A than of the other vitamins. Yet the fact that vitamin A is very unevenly distributed among our staple articles of food, while it is also very necessary to good nutrition and health, makes it important that the known facts of its occurrence be kept in mind in all considerations of the adequacy of the food supply.

Among plant products seeds are relatively poor and leaves relatively rich in vitamin A. In the seed the embryo is usually

much richer in this vitamin than the endosperm. McCollum puts these relationships in terms of the biological function of the tissue, the actively functioning cellular tissue of the leaf and the potentially active embryo of the seed being richer in vitamin A than are the storage organs such as the endosperm of the seed, or a starchy tuber, or a thick fleshy root. Steenbock and his associates have however reported as noted above, that large differences may occur within the same type of material as classified by McCollum. Thus they find maize with yellow endosperm to be much richer in vitamin A than are those varieties whose endosperm is white (Steenbock and Boutwell, 1920), yellow sweet potatoes much richer than ordinary white potatoes, and carrots than beets or white turnips. (Steenbock and Gross, 1919, 1920.) The richness of green leaves in vatimin A fits equally the hypothesis of McCollum or of Steenbock since they are rich in yellow pigment which is simply masked by green. Green peas also were found to contain much yellow pigment and to be richer in vitamin A than were less highly pigmented yellow peas. (Steenbock, Sell and Boutwell, 1921.) Unpublished results in the laboratory of one of us indicate that the green pod of the string bean is rich in vitamin A, thus resembling the leaves rather than the seeds.

Osborne and Mendel (1920c) fed equal weights (0.1 gram per rat per day) of butter fat, dried spinach, dried alfalfa leaves or dried tomato separately as sole source of vitamin A and concluded from the resulting rate and extent of growth that the dry matter of tomato or of green leaves such as spinach contains an even higher concentration of vitamin A than does butter fat. Cooper has reported the presence of vitamin A in orange peel; and Osborne and Mendel have demonstrated its presence in the peel-free juice of the orange.

Steenbock and Gross (1919) summarize their comparison of roots as sources of vitamin A as follows: "With 15 per cent of the diet made up of roots (dried) as the source of the fat-soluble vitamin we have in the case of the yellow sweet potato and carrot normal growth and even rearing of the young made possible, but in the case of the rutabaga, dasheen, red beet, parsnip, potato, mangel, and sugar beet, complete failure resulted. In fact, in some instances failure at such higher levels as were tried —25 per cent in the case of the mangel and sugar beet and 83

per cent in case of the dasheen—was also observed." Steenbock and Gross also (1920) compared cabbage, lettuce, chard and spinach as sources of vitamin A and found these to be in general richer in this vitamin than the roots, but not without exception. Cabbage appeared hardly as rich in vitamin A as did carrots and yellow sweet potatoes, though it was richer than any of the other roots tested. Lettuce contained more vitamin A than cabbage but not so much as chard and spinach, of which the spinach seemed the richest source of all the vegetables tested by these investigators. In this investigation, as in the studies of Osborne and Mendel and of McCollum, typical leaves used as hay for cattle, such as alfalfa, clover, and timothy, were found when fed dry to be very rich in vitamin A, a matter of much importance in connection with the vitamin value of cow's milk, especially that produced in winter when such hay is chiefly consumed.

Among animal products the muscle tissues (ordinary meats) are poor in vitamin A while glandular organs such as liver, and especially eggs and milk are much richer sources. Since the glandular organs constitute but a small part of the animals which must be slaughtered to obtain them, it is evident that raising animals for slaughter is an uneconomical method of obtaining a supply of vitamin A. In eggs and milk, however, we have foods which are rich in this vitamin and of which increased amounts can be produced much more economically. As we have no evidence of synthesis of vitamin in the animal body, it is probable that whatever we obtain through animal products has originally come from plants. We have seen that the food of an animal influences the store of vitamin A carried in the animal's body, but in what tissues the surplus is stored, and whether or to what extent meats and eggs are made richer in vitamin when the animal producing them is fed vitamin-rich food, has been but little studied as yet.

In the case of milk, the vitamin A content is believed to be influenced by the abundance of the supply contained in the feed. Hence the presence of an abundance of vitamin A in the grasses on which dairy cows are pastured in summer and in the hays fed in winter is an important factor in ensuring that the milk produced by the cow is rich in vitamin A at all times of the year. A further factor of safety in this connection is the

fact that the store of this vitamin carried in the body of the cow is undoubtedly drawn upon to keep up the normal concentration of vitamin A in the milk in case of fluctuations in the supply received in the feed.

Of interest in this connection is the recent observation of Drummond, Coward and Watson (1921) that colostrum has a much higher concentration of vitamin A than the later milk. They are inclined to regard this higher value of colostrum as an indication of a mobilization of the reserves of the mother since it does not appear to be proportional to the fat content. "It is again interesting to recall that there is also a partial mobilization of the lipochrome pigments of the mother's body fat for the production of colostrum which normally contains a much higher concentration of those coloring substances than the later milk." A similar relation between vitamin A and the lipochrome pigments is suggested in the apparent tendency reported by these investigators for milk from cows of the Jersey and closely related breeds to be richer in vitamin A than that of Shorthorn or Black Angus. As a possible explanation it is suggested that cows of the former breeds may have a higher storage capacity for vitamin A similar to their higher capacity for storing pigment.

It is important to note that milk contains much more of the "fat-soluble" vitamin than is contained in its fat globules. According to a brief statement made by McCollum the vitamin A in a given volume of milk is about equally divided between the fat globules and the aqueous portion. This would mean that skimmed milk will contain about half as much vitamin A as whole milk, and dry skimmed milk will be about one-third as rich in vitamin A as is butter fat. Sherman, MacLeod and Kramer (1921) while not measuring quantitatively the relative amounts in whole and skimmed milk have confirmed the fact that skimmed milk is an important source of vitamin A, though of course by no means comparable with whole milk in this respect.

In general it would seem that milk in all forms, eggs, butter and green vegetables are the richest food sources of vitamin A, followed by some roots, the embryos of seeds and the glandular organs of animals, then by roots and seeds generally and muscle tissue meats and finally by artificially refined products such as

white rice, patent flour, sugar and many of the refined fats which are practically devoid of the vitamin. McCollum has repeatedly emphasized the fact that diets composed essentially of breadstuffs, meats, sweets, tubers, and many fats are likely to contain too little of this vitamin and he has classified milk and green vegetables as "protective foods" because of their richness in vitamin A as well as in calcium.

Until we have more precise knowledge regarding the destruction of vitamin A by heat and oxidation it would be premature to attempt broad generalizations in regard to whether or not significant losses of vitamin A are likely to occur when foods are preserved, stored or cooked. Under ordinary normal conditions, however, it seems reasonable to believe that such losses are not likely to be large. Steenbock and Boutwell (1920a) found no demonstrable loss of vitamin A when chard, carrots, sweet potatoes, squash, or yellow maize was heated for 3 hours at 15 pounds pressure at 120° C. in an autoclave. In butter it may be more susceptible to heat but when conditions are such as not to injure the flavor it is probable that the vitamin value is not materially injured. McCollum (1918) reports a high vitamin A value in the fat of canned evaporated milk, and the same has been found to be true of ripened cheese. Dried milk has repeatedly been used in different laboratories as sole source of vitamin A with entire success, and even when a relatively small proportion of cooked or dried milk has been depended upon there has been no evidence of its having lost any significant proportion of the vitamin A of the original fresh milk (Sherman, Rouse, Allen and Woods, 1921). In general it seems probable that foods known to be good sources of vitamin A as tested, will still be good sources after the ordinary industrial or domestic manipulations or storage.

Drummond, Coward and Watson (1921) from an extensive examination of storage butter concluded that the season at which the butter is placed in storage, i. e., the feed of the cows at that season, is a more important factor in determining the value of the storage butter as a source of vitamin A than the length of time the butter remains in storage provided undue exposure to air is prevented and other conditions are good. As under present conditions by far the largest part of the butter placed in storage is that produced when the cows are on green pasture and

are consequently giving a greater yield of butter of superior quality and as it is kept in wooden casks or tins during storage, it seems reasonable to assume that such butter may be scarcely less rich in vitamin A than fresh butter and certainly of higher vitamin content than renovated butter. Thus the development of free acidity in butter may occur without appreciable loss of vitamin A if oxidation is prevented, while in so far as the process of renovating rancid butter offers opportunity for oxidation it may entail sufficient losses of vitamin A to render the product of materially less value in this respect.

Of interest in this connection is the statement made to one of the writers by an expert judge of butter ("butter taster") that in the winter months storage butter is to be preferred to much of the fresh butter obtainable at the time, on account of its summer flavor.

Reported Occurrence of Vitamin A.

Food Material	*Literature Reference*
Alfalfa	McCollum, Simmonds and Pitz 1916b; Osborne and Mendel 1919; Steenbock and Gross 1920; Steenbock and Boutwell 1920b; Osborne and Mendel 1920c.
Beans	
soy	Daniels and Nichols 1917; Osborne and Mendel 1917c.
string	Sherman et al, unpublished.
velvet	Sure and Read 1921.
Beef fat	Osborne and Mendel 1915a; Halliburton and Drummond 1917; Steenbock, Sell and Buell 1921.
Butter	McCollum and Davis 1913, 1914, 1915; Osborne and Mendel 1913, 1913a, 1915a, 1916, 1920c; McCollum and Kennedy 1916; McCollum, Simmonds and Steenbock 1917;

Food Material	*Literature Reference*
Butter	Stephenson 1920; Hopkins 1920a; Zilva 1920a; Drummond and Coward 1920a and b; Steenbock, Sell and Buell 1921; Zilva and Miura 1921; Drummond, Coward and Watson 1921; Aron and Gralka 1921.
Cabbage	McCollum, Simmonds and Pitz 1916b; Delf 1918; Delf and Skelton 1918; Osborne and Mendel 1919, 1920b; Zilva 1920; Coward and Drummond 1921.
Carrots	Denton and Kohman 1918; Steenbock and Gross 1919; Zilva 1920; Osborne and Mendel 1920c; Steenbock and Boutwell 1920a, 1920b; Stephenson 1920; Coward and Drummond 1921.
Carrot tops	Sherman, unpublished.
Chard	Steenbock and Gross 1920; Steenbock and Boutwell 1920a.
Cheese	Sherman, unpublished.
Clover	Osborne and Mendel 1919, 1919d, 1920c; Steenbock and Gross 1920.
Coconut meal press cake	Johns, Finks and Paul 1919. Jansen 1920.
Codliver oil	Osborne and Mendel 1914; Zilva and Miura 1921; Steenbock, Sell and Buell 1921; Aron and Gralka 1921.

Food Material	*Literature Reference*
Cod testicle fat	McCollum and Davis 1915.
Corn (maize)	McCollum and Davis 1915a; McCollum, Simmonds and Pitz 1916d; Steenbock 1919; Steenbock and Boutwell 1920, 1920a; Drummond and Coward 1920a.
green shoots	Coward and Drummond 1921.
Cottonseed	
flour	Richardson and Green 1916, 1917a.
meal	Osborne and Mendel 1917.
oil	Daniels and Loughlin 1920; Drummond and Coward 1920a.
Egg yolk	McCollum and Davis 1913; Osborne and Mendel 1913a; McCollum and Davis 1914b; McCollum 1916; Aron and Gralka 1921.
Fish	Drummond 1918a.
Heart (pig)	McCollum and Davis 1915a; Osborne and Mendel 1918a.
Horse fat	Drummond and Coward 1920a.
Kidney (pig)	McCollum and Davis 1915; Osborne and Mendel 1917b, 1918a.
Lard	Daniels and Loughlin 1920; Drummond, Golding, Zilva, and Coward 1920.
Lettuce	Steenbock and Gross 1920.
Liver	
pig	McCollum and Davis 1915a; Osborne and Mendel 1917b, 1918a.
beef	McCollum, Simmonds and Parsons 1921.
Margarin	Halliburton and Drummond 1917.

Food Material	*Literature Reference*
Milk	
whole raw	McCollum, Simmonds and Pitz 1916c; Dutcher, Kennedy and Eckles 1920; Hopkins 1920; Mattill and Conklin 1920; Mattill 1921; Drummond, Coward and Watson 1921.
skimmed raw	McCollum, Simmonds and Steenbock, 1917; Sherman, MacLeod and Kramer 1921.
dried	Coutts 1918; Winfield 1918; Sherman, Rouse, Allen and Woods 1921; Rosenau 1921.
condensed	Rosenau 1921.
Mutton fat	Drummond and Coward 1920a.
Oat kernel	McCollum, Simmonds and Pitz 1917.
Orange juice	Osborne and Mendel 1920d; Hess, McCann and Pappenheimer 1921.
Orange peel	Cooper 1921.
Palm oil	Drummond and Coward 1920a.
Peas	
green (*Vicia sativa*)	McCollum, Simmonds and Parsons 1919a;
yellow	Steenbock, Sell and Boutwell 1921;
green shoots	Coward and Drummond 1921.
Pig kidney fat	McCollum and Davis 1915; Drummond and Coward 1920a.
Potato, sweet	Steenbock and Gross 1919; Steenbock and Boutwell 1920a.
white	McCollum, Simmonds and Parsons 1918a; Steenbock and Gross 1919; Osborne and Mendel 1920c.
Rice, unpolished	Guerrero and Concepcion 1920.

Food Material	*Literature Reference*
Seaweed green (Ulva and Cladophora)	Coward and Drummond 1921.
Spinach	Osborne and Mendel 1919, 1919d, 1920c. Steenbock and Gross 1920.
Squash (Hubbard)	Steenbock and Boutwell 1920a.
Sunflower green shoots	Coward and Drummond 1921.
Timothy	Osborne and Mendel 1919, 1920c.
Tomato	Osborne and Mendel 1920c.
seed press cake	Finks and Johns 1921.
Turnip green shoots	Coward and Drummond 1921.
Whale oil	Drummond 1918a.
Wheat embryo	McCollum and Davis 1915a; McCollum, Simmonds and Pitz 1916.
bran	Stammers 1921.

Vitamin A in Nutrition and Health.

Some of the effects of a lack of sufficient vitamin A in the diet have already been given in the discussion of the characterization of the vitamins, and the reasons for our belief in the independent existence of at least three of them. These effects may now be summarized briefly as a basis for our consideration of the broader significance of this vitamin in the normal physiology of nutrition and reproduction and the ability to resist disease.

When a diet lacking vitamin A but adequate in all other respects is fed to a young growing rat, growth may cease, or continue slowly, or may continue for as much as 10 weeks at a normal or nearly normal rate, depending upon the store of vitamin A in the body of the experimental animal at the begin-

ning of the experiment and the amount of the experimental diet eaten. Under favorable conditions growth continues for some time at a nearly normal rate (as indicated by the curves shown in Fig. 9 above) during which time the surplus of vitamin A possessed at the beginning has presumably become exhausted. Growth then slackens and usually soon ceases; often a rather rapid lose of weight begins shortly and the animal goes into a condition of general decline leading to death. Sometimes, perhaps because of unusual stamina or appetite or because he receives unrecognized small amounts of vitamin A in the experimental diet, the animal remains at nearly constant weight for several weeks.

By the time the body's reserves of vitamin A have been exhausted and growth ceases, the animal becomes more susceptible to bacterial infection, and this lowered resistance often shows itself in various ways—most conspicuously in the tendency of a large proportion of such experimental animals to develop a characteristic disease of the eye, variously known as ophthalmia, xerophthalmia, keratomalacia, conjunctivitis, or keratoconjunctivitis. This usually begins with a swelling of the lids of one or both eyes or with indications that the eye is becoming unduly sensitive; then there commonly develops an inflamed and catarrhal condition of the conjunctivæ with a bloody or purulent discharge, the lids becoming scabby or sticky. This with the swelling of the lids sometimes results in the eye being found completely closed. Often the inflammation extends to the cornea and if not treated may result in permanent blindness, though the animal often dies before the eye disease reaches this stage. The typical eye condition undoubtedly involves infection and in this sense is not purely a deficiency disease, yet it is essentially so, inasmuch as the dietary deficiency so enormously increases the susceptibility of the animal to the infection as practically to determine the occurrence of the disease. It is also very significant that without any other treatment whatever the eye disease, if not too far advanced, usually disappears quickly when the animal is given any food containing a sufficient amount of the vitamin A.

The relation of the diet to this eye disease and its cure was discovered by Osborne and Mendel in 1913. At that time they stated that a "type of nutritive deficiency exemplified in a form

of infectious disease prevalent in animals inappropriately fed is speedily alleviated by the introduction of butter fat into the experimental rations," and in 1914 they reported "uniform success by substituting codliver oil for a portion of the lard in our standard diets. . . . Not only was growth resumed in most cases at a very rapid rate, but all evidence of malnutrition, especially the affection of the eyes, promptly disappeared." Similar cures by feeding materials containing vitamin A have been reported repeatedly by Osborne and Mendel and others since that time so that there can be no doubt of the frequent cure of the disease by dietary treatment alone. Moreover, its cure by any other than dietary means is doubtful, if allowance be made for the fact that on diets only partially deficient in vitamin A the animals not infrequently show the eye trouble in some degree and recover spontaneously. Bulley (1919) believed the eye trouble to be an infection controllable by sanitary precautions and local treatment with antiseptics, but Osborne and Mendel (1921) found this not to be true in their cases, and Stephenson and Clark (1920) have pointed out that the diets used by Bulley may not have been so thoroughly freed from vitamin A as they were intended to be. In their own experience Stephenson and Clark report the development of the eye disease in 13 of 46 rats kept on diets deficient in vitamin A. Emmett (1920) found it in 120 out of 122 such cases and in no case on other diets. Osborne and Mendel (1921a) in a recent full discussion of the subject give the results of an examination of their laboratory records of 1000 rats representing essentially the entire group under study in their laboratory during one year. The rats were classified according to diet and the total numbers and numbers showing eye symptoms were counted with the following results:

Incidence of Eye Disease in Osborne and Mendel's Rats. Nos. 5000-5999.

	Total number of rats.	*Number with eye symptoms.*
On diets deficient in Vitamin A	136	69
On diets deficient in Vitamin B	225	0
On diets otherwise deficient	90	0
On diets experimental but presumably adequate	201	0
On mixed food (stock animals)	348	0
	1000	69

"From this summary it will be seen that although nearly one-half of the thousand rats were on diets undoubtedly deficient, not a single case of the eye disease was observed in animals other than those experiencing a deficiency in fat-soluble vitamin in the ration. The incidence of the disease among this group is 50 per cent, somewhat higher than that observed by Stephenson and Clark, and lower than that reported by Emmett. It should be added that in observations on several thousands of rats we have never observed distinct symptoms of comparable eye disease in any animals except those which had experienced a deficiency of fat-soluble vitamin in their diet."

The eye disease sometimes appears before complete cessation of growth occurs. Most frequently the appearance of the eye disease very nearly coincides with the period in which the rat ceases to grow and begins to lose weight. Of the 69 cases above reported by Osborne and Mendel, 33 showed eye symptoms at approximately maximum body weight; 15 after a decline of 10 to 20 grams from the maximum; 13 after a loss of 20 to 30 grams; 8 after a loss of more than 30 grams in body weight. The pathology of this eye disease has been studied by Wason (1921).

As the animals approach maturity their requirement for vitamin A becomes relatively less as indicated by the fact that they can remain in health for a longer time upon a diet deficient in this respect. In how far this means a real diminution in the vitamin A requirement and in how far it depends upon the fact that the older and larger animal, if it has previously been well fed, has a larger store upon which to draw in such emergencies is not yet clear; and the solution of the question is complicated and difficult because our usual criteria become less serviceable as the young animal approaches maturity since growth has now nearly ceased and it is reasonable to suppose that the conjunctival tissue will be less sensitive in an animal which has made a normal growth and development than in the young growing individual. Full grown rats when placed upon diets lacking the A vitamin are less likely to develop the characteristic eye trouble than are young growing individuals, but if the experiment is sufficiently prolonged eye trouble appears in a considerable proportion of cases even of rats which had grown to maturity on good diets.

The relation of the eye disease to this dietary deficiency (lack of vitamin A) has been experimentally demonstrated in other species as well as rats (Nelson and Lamb, 1920; Steenbock, Nelson and Hart, 1921—Figs. 11 and 12), and special interest attaches to the question as to whether the use of dietaries poor in the A vitamin may be a factor in the susceptibility to eye trouble in man. McCollum (1918) writes "There are several instances of the occurrence of conditions described in the literature as xerophthalmia, which seem to be beyond question, cases in which the disease has occurred in man as the result of specific starvation for the dietary essential, fat-soluble A." The reports here referred to are those of Mori (1904) in Japan whose cases of xerophthalmia occurring among children at a time of food shortage could be cured by feeding chicken livers (liver being rich in vitamin A) and those of Bloch (1917) whose cases among the children of the Danish poor also responded to the feeding of foods rich in the A vitamin. Wells found in Roumania, and Dalyell in Vienna, cases of children suffering from eye trouble which could be cured by codliver oil and presumably arose from a deficiency of vitamin A in their food (Blunt and Wang, 1921) and recently McCollum, Simmonds and Parsons (1921) have attributed "night blindness," an eye trouble of frequent occurrence in Northern regions, to the use of diets poor in this vitamin. Appleton (1921), on the other hand, appears to doubt the dietary origin of night blindness.

The observations of Bloch referred to above, as well as some later investigations by the same author, have recently been published in English (Bloch, 1921) under the auspices of the British Committee on Accessory Food Factors, on account of their unusual interest when considered in conjunction with the experimental production of the disease in rats by a deficient diet. The xerophthalmia observed among these Danish children was regarded as one phase of their condition of malnutrition. The eye disease appears to have been quite definitely related to deficiency of vitamin A in the diet since it was cured by feeding with whole milk or with codliver oil and in 1918 the disease nearly disappeared from the community coincidently with the general introduction of butter into the dietary of the poorer people, this being brought about through government food regulations. Seasonal variation of the incidence of the disease was

FIG. 11.—(Upper photograph.) Ophthalmia in dog as the result of diet deficient in vitamin A. (Steenbock, Nelson and Hart, 1921.)

FIG. 12.—(Lower photograph.) Same dog shown in Fig. 11 cured in 10 days by the addition to his diet of 20 cc cod-liver oil per day. (Steenbock, Nelson and Hart, 1921.) (By permission of the *American Journal of Physiology*.)

studied and it was found to be most prevalent in the season at which children make their most rapid growth. "As xerophthalmia is caused by the absence of something essential to growth, it is logically to be expected that the disease will predominate during the part of the year when the organism consumes the largest quantity of this lipoid material (vitamin A) for its growth, as is here found to be the case. It is also suggested that the specific lipoid (vitamin A) may be necessary for the formation of antibodies against infection and may be continually used up in this process as well as in growth."

That lack of vitamin A may lead to weakness or abnormality of other tissues as well as those of the eye, has been shown by the work of several investigators. Osborne and Mendel (1921a) refer to diarrhea and diminished appetite as frequently resulting from this lack; and they have definitely correlated it (1917d) with the occurrence of phosphatic renal calculi among their experimental animals. McCollum (1917) and also Drummond (1919b) report increased susceptibility to infections of the respiratory system.

Steenbock, Sell and Buell (1921) confirm this and suggest that the failure to develop the characteristic eye disease in some cases may be due to a measure of immunity acquired through the respiratory infection. As symptoms of the respiratory infection, the incidence of which is regarded by these authors "as part of the syndrome induced by fat-soluble vitamin deficiency," they mention "a nasal or bronchial catarrh or even pulmonary infection with mucous or purulent exudate, at times even resulting in hemorrhage. Animals thus afflicted in the early stages of the disease sneeze and cough violently but later as the inflammation becomes confined more to the lungs, the cough subsides, and dyspnea becomes very pronounced with the slightest activity."

In agreement with other observers, these authors have also noted evidences of cutaneous malnutrition in animals deprived of vitamin A. Such affections are more usual in rats over four months old and may take the form of scabbiness of the tail or ears, sores or abnormal growths on the nose, thin and bushy hair, and sore feet.

It is suggested that rats on diets suspected of being deficient in vitamin A should be observed for all such symptoms as well

as for eye disease and failure of growth, and that judgment be based on the sum total of indications (Steenbock, Sell and Buell, 1921).

Vitamin A and rickets.—E. Mellanby (1919) in an extended study of experimental rickets in puppies obtained results for the most part in keeping with the view that rickets is due primarily to a deficiency of vitamin A. This view was adopted, although with some reservation, in the British Committee Report (Hopkins, Chick, Drummond, Harden and Mellanby, 1919) and has been so completely accepted by some as to lead them to speak of vitamin A as the "antirachitic vitamin." It is certain, however, that vitamin A does not bear any such simple and direct controlling relation to rickets as does vitamin B to beriberi or vitamin C to scurvy. Sherman and Pappenheimer (1921, 1921a) showed that rickets may be caused or prevented by changes in the mineral elements of the food without any alteration of either the protein or the vitamin components of the diet, and this has been confirmed by Shipley, Park, McCollum, Simmonds and Parsons (1921). Furthermore, Hess, McCann and Pappenheimer (1921) have found that deficiency of vitamin A in a diet otherwise adequate does not lead to rickets. It is quite possible that rickets may develop more readily upon diets poor in vitamin A than on those which contain it in abundance. But the mineral elements evidently bear a more direct relation to the disease than does the vitamin and it is certainly misleading to speak of vitamin A as "the antirachitic factor" of the diet.

The subject of rickets is still under active investigation and therefore can be discussed only in tentative terms. Since the preceding paragraph was written, McCollum, Simmonds, Shipley and Park (1921a), in a further discussion of their own results and those previously published by Sherman and Pappenheimer. conclude that both the ratio between calcium and phosphorus and the proportion of vitamin A or some related substance are factors in the production of rickets. Regarding the vitamin factor they have found codliver oil effective in preventing and curing rickets in doses in which butter fat is not. If this difference between codliver oil and butter fat is qualitative and not merely quantitative, then either "vitamin A" stands for different substances in the two cases or codliver oil contains an antirachitic as well as an antixerophthalmic substance. On this

point McCollum, Simmonds, Shipley and Park say, "We are at present unwilling to commit ourselves to the view that the anti-xerophthalmic factor and the factor concerned in the ossification and growth of the skeleton are distinct, though we are prepared to acknowledge that such may be the case. We, therefore, use fat-soluble A to describe both the antirachitic and the anti-xerophthalmic factors, assuming that the former has a separate existence." They conclude (1921b, p. 508) that their "experiments showed that it was possible to produce a pathological condition in the rat unquestionably similar to the rickets of the human being through the diet alone. In the second place, they showed that rickets could be induced by means of a ration the faults of which were clearly defined and sharply limited, viz., deficiencies in phosphorus and fat-soluble A. In the third place, they indicate that deficiency of phosphorus in the ration insufficiently supplied with fat-soluble A would give rise to rickets only when calcium was present in a ratio considerably higher than the calcium-phosphate ratio which is optimal for ossification."

Korenchevsky (1921) has reported that diets deficient in calcium alone produce changes in the skeleton of the rat resembling but not identical with rickets and that these changes are more marked when the diet of the mother during lactation has also been deficient in calcium. Deficiency in vitamin A caused changes similar to rickets and in some cases typical of rickets, the latter being true only of rats whose mothers had also been fed on diet deficient in vitamin A during pregnancy and lactation. Changes typical of rickets occurred most frequently on diets deficient in both calcium and vitamin A. Korenchevsky is of the opinion that "vitamin A is apparently closely related to the metabolism of calcium in the organism particularly in the bone."

Dalyell and Chick (1921) and Hume and Nirenstein (1921) report an apparent correlation between lack of vitamin A and the "hunger-osteomalacia" so prevalent in Vienna at the close of the World War. In the dietetic treatment of this condition little improvement resulted from increasing the energy value of the meager diet consisting chiefly of bread and sauerkraut by the addition of cereals or sugar but rapid recovery followed the addition of fats rich in vitamin A particularly codliver oil. These investigators also note the increase in Vienna of rickets

in children and late rickets in young adults simultaneously with the occurrence of hunger-osteomalacia and suggest that lack of vitamin A may be an important factor common to these disorders.

In this connection however we must not fail to keep in mind the facts, established by recent American work, that lack of vitamin A does not of itself cause rickets, the more essential cause being a disproportion between the calcium and the phosphorus of the food; and that this disproportion may be due to an absolute or a relative deficiency of either calcium or phosphorus, the histological results being apparently somewhat different in the two cases.

Meantime Huldschinsky (1920) and Hess and Unger (1921a, 1921b, 1921c) emphasize the potency of ultraviolet rays and direct sunlight in the prevention and cure of rickets and suggest that the marked seasonal variation in the incidence of rickets may be due to the fact that lack of sunlight is a larger factor in bringing about the disease than is any dietary deficiency.

An hypothesis consistent with all these observations would be that a low intake of phosphorus with faulty ratio of calcium to phosphate creates a tendency toward rickets, which, however, may be prevented or cured either by a sufficient amount of vitamin A or by adequate exposure to direct sunlight or to ultraviolet rays.

Relation to dentition.—M. Mellanby (1918) showed that diet has a marked effect upon the development of the teeth in puppies, and the nature of the diets used makes it appear probable that vitamin A was an important factor in the results obtained. As summarized by the British Committee, "Apparently the formation of calcified enamel and the adequate spacing of the teeth in puppies is dependent on an abundance of some such factor. In human beings calcification of the teeth is a much slower process and continues till the eighteenth year. In order to ensure perfect calcification of the teeth, therefore, it is necessary that the diet should contain adequate supplies of the accessory food factor up to this time, and a deficiency at any period will be reflected in a corresponding defect in the formation of the teeth."

Relation of vitamin A to general vigor.—As the result of his extended investigation of the dietary properties of the various

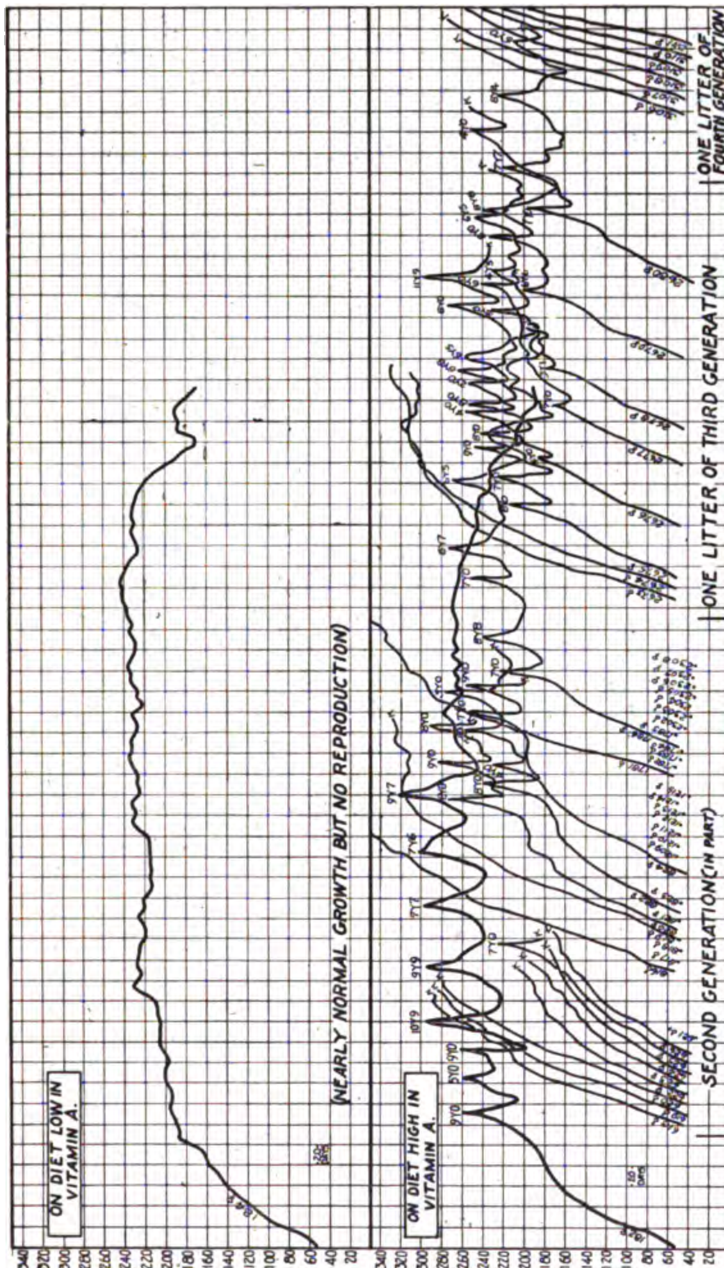


FIG. 13.—Experiments illustrating the importance of quantitative differences in the intake of vitamin A. Rat 184, upper section of the chart, received a diet of whole wheat and dried skimmed milk furnishing sufficient vitamin A for practically normal growth but not for reproduction. Rat 182, lower section of chart, a twin sister of 184, received a diet differing only in that it contained whole instead of skimmed milk. There was little difference in growth but marked difference in vigor as indicated by the capacity for reproduction and successful suckling of the young. (Sherman and MacLeod, unpublished data.)

types of food, McCollum concludes that dietaries deficient in vitamin A and in calcium content are of relatively frequent occurrence; that such deficiencies lower the vigor of the body and its ability to resist disease; and that as the result of such weakening there develops an increased susceptibility so that the incidence of any of a number of diseases such as tuberculosis and pellagra may be largely influenced by the adequacy of the diet as regards vitamin A.

Whether the relation of vitamin A to these diseases proves to be important or not, experiments upon laboratory animals have already quite definitely shown that general vigor as exhibited not only in growth but also in capacity for reproduction and successful suckling of the young, is dependent in large measure upon the amount of vitamin A in the food. Drummond (1919b) stated that a liberal allowance of vitamin A resulted in better reproduction, and McCollum as well as Steenbock and their associates have repeatedly published implied confirmation of this finding by reporting cases in which on diets which were being tested for vitamin A the amount of this factor appeared adequate for growth but not for reproduction, or for reproduction but not for rearing of the young. Direct comparisons of dietaries otherwise identical but differing in their content of butter fat or by the substitution of butter fat by lard, are showing that such differences in the vitamin A contents of two diets, even though both are adequate for growth and apparently for general health, may have a most marked influence upon the capacity to produce and rear young (Sherman and MacLeod, unpublished data, see Figs. 13 and 14).

By means of the methods developed by Evans and his coworkers in the Department of Anatomy of the University of California, Evans and Bishop (1922) have thrown further light upon the significance of vitamin A in relation to reproduction. While the details of their work have not been published at the time this is written, we are enabled by the courtesy of Dr. Evans to quote the following advance abstract prepared by him:

"Studies herein reported confirm the impression that rats may be successfully reared on diets poor in vitamin A if the diet is not too deficient in this essential. They may for months grow normally and not suffer from the so-called xerophthalmia. It must be admitted that we have not had previously a method

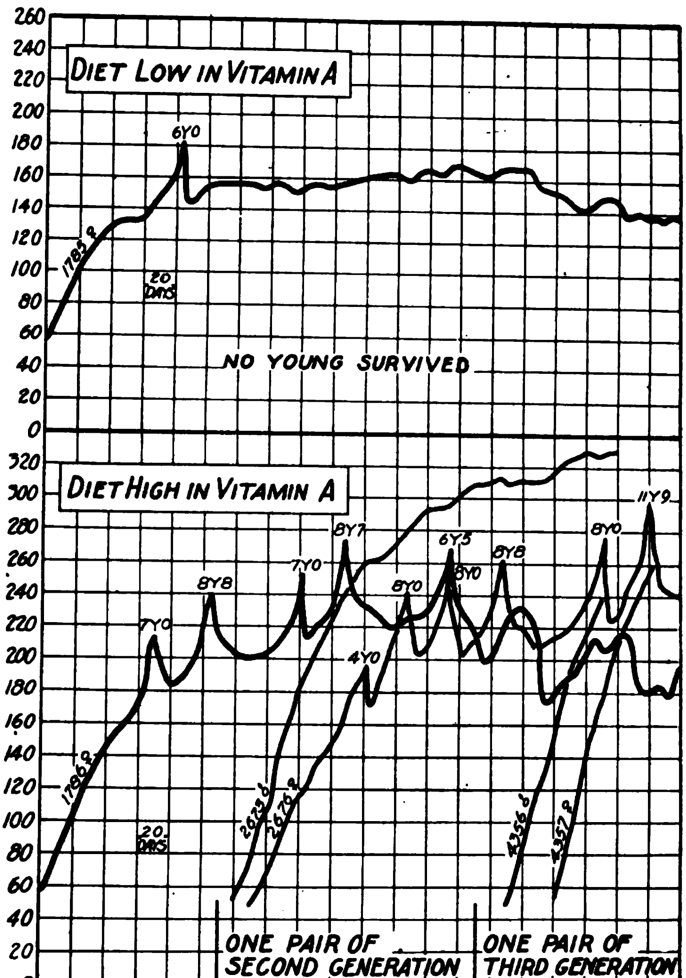


Fig. 14.—Showing comparison of diets containing different amounts of vitamin A similar to the comparison shown in Fig. 13 except that in this case the diets consisted of whole wheat, dried skimmed milk and lard or butter respectively, so as to contain the same proportion of fat and differ only in fat-soluble vitamin contained. Again there was little difference in growth but the diet containing the higher proportion of vitamin A resulted in a much higher degree of vigor as shown by capacity for reproduction and successful suckling of young. In this case only one pair each of the second and third generations is charted. (Sherman and MacLeod, unpublished data.)

for the detection of deficiencies in this vitamin which, nevertheless, permit fair growth and apparent health. Studies on the oestrous cycles of such rats show that they may suffer from an invariable and continuous abnormality or disfunction of the ovaries.

"It has previously been shown in this laboratory that ovulation in the rat can be detected in the living animal by a series of histological changes in the vaginal smear, changes which are correlated with the growth, maturation and rupture of the Graafian follicles at periodic intervals. When for any reason the follicles are unable to completely mature (as in animals treated with hypophyseal substance) vaginal oestrous changes are absent. A totally different picture is produced if follicles develop but are unable to rupture; under such circumstances, the oestrous changes may be remarkably prolonged, and the dioestrous pause in fact obliterated. As Evans and Long have shown, this occurs as a rare anomaly in large colonies of animals. But this prolongation of oestrous vaginal changes and failure of ovulation occurs in 100 per cent of animals reared on diets which are low in vitamin A but which have nevertheless permitted preliminary normal growth. We have used typical diets employed by E. V. McCollum and by T. B. Osborne and L. B. Mendel, in which the chief fat content was furnished by lard. On the administration of small quantities of dried, powdered leaves of young succulent alfalfa or of small quantities of butter fat, this characteristic abnormality in oestrus and ovulation was cured."

Reynolds and Macomber (1921) have also found a decrease in fertility to result from a paucity of vitamin A in the diet.

The vitamin A which the body stores when well fed probably constitutes a very important resource which can be drawn upon to enable the organism to meet either the normal increased demands of reproduction and lactation, or emergencies such as subsequent dietary deprivations. The survival periods of mature rats when placed upon food deficient in vitamin A with or without other dietary deficiencies have been found to vary greatly according to the nature of his previous dietary and especially its richness in vitamin A (Sherman, MacLeod and Kramer, 1920; Sherman and Kramer, unpublished results).

While the need of vitamin A is most readily demonstrated

upon young animals, Drummond (1919) has found that the adult animal organism requires a supply of vitamin A which apparently need not be so large as for the young growing animal but which nevertheless is an important factor in the maintenance of health. "It appears probable that the resistance to diseases of bacterial origin is seriously impaired by a failure of the animals to obtain a sufficient supply of the fat-soluble factor. There is therefore every reason that great care should be taken to ensure that dietaries of adults contain an adequate supply of foodstuffs in which fat-soluble A is present."

While several writers have emphasized the conspicuous relation of vitamin A to growth and the decreased need of the adult, some even raising the question whether vitamin A is necessary to adults or not, it has been suggested by Sherman, MacLeod and Kramer (1920) that the apparent independence of the adult as regards the vitamin A content of his daily food may really be due in large measure to the store of this vitamin carried in the body of an adult whose food has normally contained a liberal amount of this substance. In accordance with this view it is found quite consistently in a considerable number of unpublished experiments that if normal adults of the same age and origin but whose previous food has been different are placed upon the same diet deficient in vitamin A, the survival period is materially longer for those whose previous diet had been richer in vitamin A and thus had enabled them to lay up a larger store.

Since the body is evidently able to store relatively much more of vitamin A than of the other vitamins, and since this store is probably a very important safeguard and resource, it seems wise that those who can afford it should invest rather liberally at all ages in food rich in vitamin A, knowing that in this case the body will store the surplus to an extent and with an efficiency which is not to be expected in the case of most other nutrients.

Summary of Properties of Vitamin A.

Vitamin A is also known as the fat-soluble vitamin, fat-soluble A, the antixerophthalmic vitamin, or sometimes as "anti-rachitic" vitamin. ?

It is distinguished from vitamins B and C both by its solubilities and by its physiological effects.

Vitamin A was first discovered in butter fat, egg yolk fat, codliver oil, and the body fat of beeves. In these animal sources it occurs dissolved in the fat (hence "fat-soluble A") and is extracted along with the fat by fat-solvents. In plant materials vitamin A is not necessarily associated with fats and not always extracted by fat-solvents, the results of different investigators being somewhat at variance on this latter point. While its solubility in ether is thus variously reported, all observers agree that vitamin A is soluble in alcohol, and, at least as found in the animal body, is soluble in fats. In general, the solubilities of vitamin A would place it among the lipoids and it undoubtedly has been responsible for at least a part of the nutritive efficiency sometimes attributed to lipoids in the past. All attempts to identify vitamin A with any known lipoid, glyceride, or fatty acid have, however, failed and there is no evidence to identify it with any chemically known substance.

To explain his observations that vitamin A as found in animal products is freely soluble in fats and dissolved with them by ether, while as it occurs in plant products it appears much less soluble, McCollum suggested the hypothesis that this vitamin exists in the plant in a state of chemical combination in which it is not soluble (or at least not freely soluble) in fats and fat solvents, but which is resolved by the animal digestive juice setting free the vitamin A which thereafter remains free and fat-soluble in the animal body and the products derived from it. Osborne and Mendel however extracted vitamin A along with some fat and coloring matter, directly from dried spinach by means of U. S. P. ether.

Several observers report that vitamin A is not destroyed when fats containing it are saponified by means of non-aqueous caustic alkali; and it has been inferred from this that it is not an ester, which in our opinion does not necessarily follow, although it may be true. Vitamin A dissolves to some extent in water, though according to McCollum's estimate it is 30 times more soluble in fat than in water.

While thus differing sharply from vitamins B and C in its much greater solubility in fat than in water, it appears to stand in an intermediate position between B and C as regards its stability to heat, and to resemble C rather than B in its susceptibility to oxidation.

Zilva found vitamin A destroyed by ultraviolet light under conditions which did not destroy B or C. Recently, however, he appears to feel that this destruction may have been due to oxidation of vitamin A by the ozone which was formed and came in contact with the vitamin-containing fat during the exposure to the ultraviolet light.

Feeding experiments show vitamin A to be entirely distinct from vitamins B and C since it will not take the place of B in preventing beriberi in pigeons or promoting growth of rats, nor of C in preventing scurvy and promoting growth of guinea-pigs.

Vitamin A is essential to growth and has sometimes been called the growth-promoting vitamin. When the intake of vitamin A is inadequate, not only is growth inhibited after a time, but there also develops increased susceptibility to infection. This shows itself conspicuously and characteristically (but not invariably) in the development of the eye disease variously known as ophthalmia, xerophthalmia, conjunctivitis, and keratomalacia.

The weakening of the body by lack of vitamin A is not confined to the tissues of the eye and it is more or less generally believed that a lack of this vitamin is a factor in such diverse diseases as renal calculus, rickets, pellagra and tuberculosis.

The amount of vitamin A in the food has also been found to have a marked influence upon the capacity for reproduction and successful suckling of the young.

In plants the green leaves, and among foods of animal origin milk and its products and eggs, are the most important sources of vitamin A. The glandular organs contain more than the muscles and the germs of seeds contain more than the endosperm.

The occurrence of vitamin A has been correlated with metabolic activity by McCollum and with carotinoid pigment by Steenbock.

So far as known the animal body does not synthesize vitamin A. The body has, however, evidently a much greater capacity for storage of vitamin A than of vitamin B or C.

The vitamin A thus stored in the body appears to be an important factor in its general stamina and ability both to resist disease and to meet the demands of reproduction and lactation.

Since vitamin A is so important both in health and disease,

and since any surplus received in the food can be stored in the body for future use, it appears wise that foods known to be rich in vitamin A, notably milk in its various forms and the green vegetables, should be used in the diet as liberally as is practicable.

Chapter V.

Vitamins in the Problem of Food Supply.

In the preceding chapters we have attempted to set forth the most important of our present knowledge of each of the three generally recognized vitamins in turn. It is felt that in conclusion a short chapter should be devoted to the place of the vitamins in the practical problem of providing an adequate and economical food supply.

While as yet we know neither the exact chemical nature of the vitamins nor precisely the manner in which they exert their physiological effects, yet sufficient data regarding their distribution in foods and their significance in nutrition are now at hand to make possible an intelligent use of food so as to provide adequately for our vitamin needs along with our other nutritional requirements, and without going beyond the range of our ordinary staple articles of food.

Health is defined as "Soundness of body; that condition of a living organism and of its various parts and functions which conduces to efficient and prolonged life; a normal bodily condition. Health implies also, physiologically, the ability to produce offspring fitted to live long and to perform efficiently the ordinary functions of their species." (Century Dictionary.)

It will be noted at once that this whole broad conception of health is included within the standards by which we now judge the nutritive value of a food or a ration in feeding experiments with laboratory animals.

Since nutritional requirements as stated in chemical terms appear to be the same for all species of animals which have been studied, it is safe to infer that in the same sense the essentials of adequate food supply are the same for mankind the world over. These requisites are: sufficient amounts of digestible organic nutrients to yield the necessary number of calories of energy; enough protein of suitable sorts; adequate amounts

and suitable proportions of a number of mineral or inorganic elements; and enough of each of at least three kinds of food hormones or vitamins.

From one point of view it is perhaps unfortunate that the energy value of food and the energy needs of the body are expressed in terms of calories, because the body is not a heat engine. The steam engine analogy so often employed to illustrate the functions of food, needs refurbishing not only because it is threadbare but also because it is both incomplete and misleading. Somewhat better may the body be likened to a gasolene engine, in which the motor energy comes not from heat but more directly from the chemical reaction involved in the explosive oxidation of the fuel, the heat being a by-product or end product of the motor energy and not its antecedent as in the heat engine.

If, then, one employs the analogy of the gasolene engine, the organic nutrients, fats, carbohydrates and proteins correspond to the fuel; the proteins and some of the mineral matters to materials of which the motor is made; other mineral matters to the lubricant; and the vitamins to the ignition sparks whose own energy is insignificant, but without which the engine cannot run however fine the material of which it is built or however abundant and appropriate the supplies of fuel and of lubricant.

The amounts of the various nutrients which are needed daily by the body for its normal nutrition have now been sufficiently studied, so that in the main each "requirement" may be stated in more or less definite quantitative terms. Thus the total food requirement (or energy requirement) is stated in calories per man per day; the requirement for protein, calcium, phosphorus, or iron, in grams per man per day. The vitamin requirement cannot be stated in terms of actual weight of the respective vitamins, but the percentages of certain foods, rich in one or more of these dietary essentials, which suffice to make an otherwise satisfactory diet adequate for normal growth and reproduction in laboratory animals have been determined for a sufficient number of cases to enable us to take account of this factor of food value in considering the prominence which should be given to each type of food in planning an adequate and economical diet.

According to the "law of the minimum" any one of the neces-

sary factors of nutrition may become the limiting factor. As recently stated by Hopkins (1921):

"What we have actually to recognize is that each of several factors may become that which limits efficiency, and that no one of these is in any strict sense more important than any other. Normal nutrition calls for a certain minimum of each one and every one. If a diet is harmoniously balanced in a chemical sense, then indeed energy does become the sole limiting factor. Nutrition then fails, of course, only when too little of the diet is eaten to yield the essential minimum of energy. But the supply of fat may become the limiting factor, and no less that of carbohydrate. Or, again, when the supply of energy consumed is ample, with fat and carbohydrate duly adjusted, the circumstance that a single essential amino acid in one case, or a vitamin in another, is present in amount below the necessary minimum converts each of these in turn into the factor which limits utilization. Small as the necessary minimum in either case may be, unless it is reached the proper use of the rest of the diet is reduced to a degree which is proportional to the deficiency. If the deficiency be complete normal utilization is altogether impossible."

Just what prominence should be given to each type of food in the provisioning of a given family or community is, therefore, a problem calling for consideration of many factors. Even if the social and psychological significance of food be left for others to discuss, we must still keep constantly in mind both the economic and the physiological aspects of nutrition if we are to make the best use of our food supplies.

How best to draw upon the different types of food in meeting the nutritive requirement, or how best to divide the money devoted to the purchase of food, must always remain to some extent an individual problem, but the solution of the problem can be greatly facilitated by an intelligent consideration of our existing knowledge of the nutritional characteristics and economic relationships of our different staple articles and types of food.

Taking nutritive requirements in the sequence above mentioned, we may group the chief types of food according to the nutritional significance of each, somewhat as follows:

1. Grain products—economical sources of energy and protein, but not satisfactory in their mineral and vitamin content.

2. Sugars and fats—chiefly significant from the nutritional standpoint as supplementary sources of energy, although some animal fats are important sources of vitamin A.

3. Meats, including fish and poultry—rich in protein or fat or both, but, in general, showing the same mineral and vitamin deficiencies as do the grains.

4. Fruits and vegetables—varying greatly in their protein and energy values but very important as sources of mineral elements and vitamins.

5. Milk—important as a source of energy, protein, mineral elements and vitamins and possessing unique efficiency as a growth-promoting food.

The relative values of different articles of food as sources of vitamins are indicated very roughly by the accompanying table based on that of the British Medical Research Committee (Report No. 38) as modified by M. S. Rose (*Laboratory Handbook for Dietetics*, 1921) and here rearranged to approximate the order of the above classification of food materials. Dairy products not otherwise classified and eggs follow milk in the table as here arranged. The use of one or more + signs to indicate the richness of the food material as a source of the vitamin is retained in the sense in which it was used in the original tabulations, namely as "an attempt to give some idea of relative values in the absence of strictly comparable quantitative data." Numerical expressions of relative values would at present hardly be justified beyond a few foods as sources of vitamin A or B and a somewhat larger number as sources of vitamin C. The looser form of comparison for a larger number of foods is certainly more representative of present knowledge.

Distribution of Vitamins in Investigated Food Materials.

+ indicates that the food contains the vitamin.
++ indicates that the food is a good source of the vitamin.
+++ indicates that the food is an excellent source of the vitamin.
— indicates that the food contains no appreciable amount of the vitamin.
? indicates doubt as to presence or relative amount.
* indicates that evidence is lacking or appears insufficient.

Source	*A*	*B*	*C*
Grain products			
Barley, whole	+	++	—
Bread, white (water)...	?	+	—

Source	*A*	*B*	*C*
Grain Products (Continued)			
Bread, white (milk) ...	+	+	?
Bread, whole wheat (water)	+	++	?
Bread, whole wheat (milk)	++	++	?
Corn (maize), white....	—	++	—
Corn (maize), yellow ...	+	++	—
Cottonseed meal	+	++	*
Flour, white	—	+	—
Grains, sprouted	+	++?	++
Malt, green	+	++?	++
Millet	++	++	*
Oats	+	++	—
Rice, polished	—	—	—
Rice, whole grain.......	+	++	—
Rye, whole	+	++	—
Wheat embryo	++	++?	—
endosperm	—	+	—
middlings, ~~commercial~~	*	+++	—
bran	+	++?	—
whole	+	++	—
Sugars and Starches			
Glucose	—	—	—
Honey	—	+	—
Starch	—	—	—
Sugar	—	—	—
Fats and Oils			
Beef fat	+	—	—
Butter	+++	—	—
Coconut oil	—	—	—
Codliver oil	+++	—?	—
Corn oil	+?	—	—
Cottonseed oil	+?	—	—
Horse fat	+	—	—
Lard	+?	—	—

* See explanation at head of table. Each * in the table may be taken as a suggestion for further research.

Source	*A*	*B*	*C*
Fats and Oils (Continued)			
Linseed oil	—	—	—
Margarine, nut	—	—	—
oleo	+	—	—
Mutton fat	+	—	—
Olive oil	—	—	—
Oleo oil	+	—	—
Orange peel oil.........	++	*	*
Palm oil	+	—	—
Peanut oil	—	—	—
Pig kidney fat..........	++	—	—
Whale oil	++	*	—
Meats and Fish			
Brains	+	++	+?
Fish, lean	—	+	*
fat	+	+	*
Heart	+	+	+?
Kidney	++	++	+?
Liver	++	++	+
Meat (muscle)	— to +	+?	+?
Meat extract	—	—?	—
Meat, canned	—	slight	—
Roe, fish	+	++	+?
Sweetbreads	+	+	*
Fruits			
Apples	+	+	+
Bananas	+?	+?	+
Cloudberries	*	*	+++
" canned ...	*	*	+++
Cocum (dried)	*	*	+
Grape juice	*	+	+
Grapefruit	*	++	++
Lemon juice	*	++	+++
juice dried	*	++	+++
Limes	*	+	+

* See explanation at head of table.

Source	*A*	*B*	*C*
Fruits (Continued)			
Mango	*	*	+
Mulberries	*	*	+
Orange juice..........	+	++	+++
peel	+	+	++
Pears	*	+	*
Prunes	*	+	—
Raspberries	*	*	+++
" canned	*	*	+++
Tamarind, dried.......	*	*	+
Tomatoes, raw	++	+++	+++
canned	++	+++	+++
dried	++	+++	++
Vegetables			
Alfalfa	+++	+++	*
Beans, kidney	*	+++	*
navy	*	+++	—
soy	+	+++	—
sprouted	*	*	++
string, fresh	++	++	++
Beets	*	+	*
Cabbage, fresh raw	+	+++	+++
cooked	+	++	++
dried	+	++	+
green	++	++	+++
Carrots, fresh raw	++	++	++
cooked	++	+	+
Cauliflower	+	++	+
Celery	*	+	*
Cress	*	*	+
Chard	++	+	*
Cucumber	*	+	*
Dandelion greens	++	++	+
Dasheens	—?	+	+
Eggplant (dried)	*	++	*
Endive	+	*	+

* See explanation at head of table.

Source	*A*	*B*	*C*
Vegetables (Continued)			
Legumes, sprouted	*	*	++
Lettuce	++	++	+++
Onions	*	++	++
Parsnips	—?	++	*
Peas	++	++	+?
" sprouted	*	*	++
Potatoes, sweet	++	+	*
white raw	+	++	++
white boiled (15 minutes)	*	++	++
white boiled (1 hour).	*	++	+
white, baked	*	++	+
Radish	*	+	*
Rhubarb	*	*	+
Rutabaga	—?	++	+++?
Sauerkraut	*	*	—?
Spinach, fresh	+++	+++	*
dried	+++	++	*
Squash, Hubbard	++	*	*
Swede	*	++	+++?
Turnips	—?	++	*
Nuts			
Almonds	+	+	*
Brazil nuts	—?	++	*
Chestnut	*	+	*
Coconut	+	++	*
Coconut press cake	+	++	*
Filberts	*	++	*
Hickory nuts	*	++	*
Peanuts	+	++	*
Pecans	*	+	*
Pine nuts	+	+	*
Walnuts, black	*	++	*
" English	*	++	*

* See explanation at head of table.

Source	*A*	*B*	*C*
Milk			
Milk	+++	++	+ variable
condensed	+++	++	+ variable
evaporated	+++	++	— ?
dried, whole	+++	++	+ variable
dried, skim	+	++	+ variable
Skimmed milk	+	++	+ variable
Dairy products			
Butter	+++	—	—
Buttermilk	+	++	+ variable
Cream	+++	++	+ variable
Cheese	++	*	*
Cottage cheese	+	*	*
Eggs			
Eggs	++	+	+ ?
Egg white	*	*	*
" yolk	+++	+	*
Yeast			
Yeast	—	+++	—
Yeast extract	—	+++	—

* See explanation at head of table.

In the summary of nutritive requirements on pp. 205-6 we have followed the order of the scientific development of our knowledge and of the best sequence for the study of dietary needs in placing the energy requirement (calories) first and the mineral and vitamin requirements last. In the practical planning of a diet or a family food supply, however, it may often seem best to provide first of all for those foods upon which we chiefly depend for the necessary mineral elements and vitamins after which the remainder of the protein and energy requirement may be covered by almost any type of food so far as strictly nutritive needs are concerned. When this plan is followed, the one responsible for the dietary should first of all provide an adequate supply of milk, vegetables and fruit, after which breadstuffs, meats, fats and sweets may be added according to taste, purse, energy requirement and individual digestive powers.

Lusk has well said that the housewife having a family of

five to feed should buy three quarts of milk a day before she buys a pound of meat. "A quart of milk a day for every child" is a good rule easy to remember. And it may be added that at least a pint a day is desirable for every adult. The division of the money spent upon the table among the different articles and types of food will vary both with tastes and with the economic conditions. In general, the more economically one must live the more he will live upon bread or other grain products since the grains usually furnish the most calories for the cost and the satisfaction of hunger more nearly corresponds with the meeting of the energy requirement than with any other one of the factors of nutritional need. While this chapter is designed to indicate something of the practical dietary application of the facts developed in the preceding chapters, yet space does not permit of the discussion of actual meal plans. For suggestions of this nature the reader is referred to Rose's *Feeding the Family*. As a "rule of thumb" for ensuring a dietary fairly balanced as regards mineral elements and vitamins without resort to detailed planning it has been found useful to budget the expenditures for food and to see that at least as much is spent for vegetables and fruit as for meats and fish, and at least as much for milk in its various forms as for all forms of flesh food. Since this suggestion does not attempt to fix the relation between expenditures for these foods and for breadstuffs it is applicable to any level of expenditure for food.

But, as war conditions have emphasized, the problem of food supply should also be viewed from a broader standpoint than the ability of the individual household to buy what it needs for its own nutrition or to make the best use of the money which it can devote to the purchase of food. In order to make full use of the present knowledge of food chemistry, the facts which have been learned regarding the vitamins and other factors of food value should be viewed from the standpoint of the wise utilization of food resources as a means of promoting the best nutrition of the people as a whole. In the pages which follow, the chief groups of foods will be briefly reviewed from this standpoint.

Breadstuffs and Other Grain Products.

The cereals and the breadstuffs made from them constitute the staff of life almost everywhere, except in the arctic and parts

of the tropics, because the grain crops are sufficiently productive and non-perishable to furnish us our most constant and economical forms of body fuel and at the same time an important protein supply. In different countries of the temperate zone the cereals and breadstuffs furnish from two-fifths to three-fourths of the total calories consumed by the entire population and a nearly equal proportion of the protein consumed. They may also furnish important amounts of mineral elements and vitamins

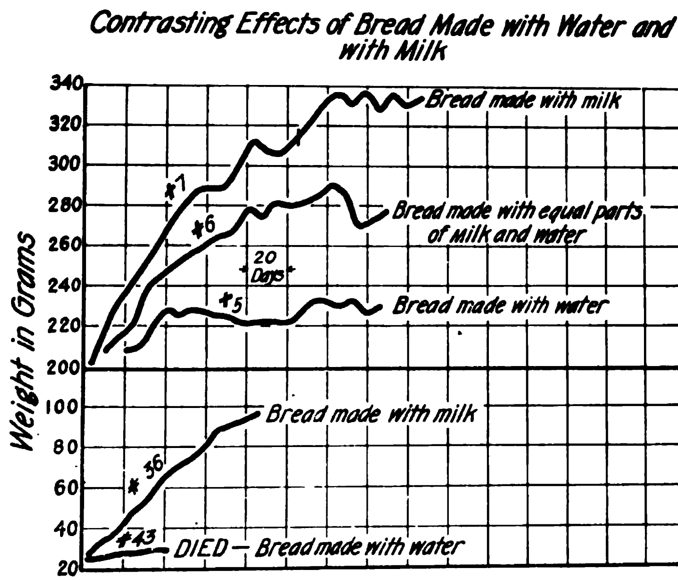


FIG. 15.—Weight curves showing marked difference in nutritive value of bread made with and without milk. (By permission of the *Journal of Biological Chemistry.*)

unless these are rejected in the milling processes, though in these respects the grains are less satisfactory than as sources of energy and protein.

Osborne and Mendel (1919) describe experiments in which whole wheat served as the sole source of vitamin B and protein, the diet consisting of whole wheat 92, salt mixture 3, and butterfat 5 per cent. This diet, fed to young rats, induced normal growth and reproduction although its protein content was only 10 per cent and this solely the protein of a single seed. Adult rats were well maintained, in Osborne and Mendel's experiments, on a diet in which the sole protein was that of whole wheat and

its proportion only 7 per cent of the weight, or 5 to 6 per cent of the total calories, of the food mixture.

Patent flour representing only the endosperm of the wheat kernel shows a much lower vitamin content than whole wheat and also a less efficient protein mixture. Its proteins are very efficiently supplemented by those of milk. Even a small proportion of milk constituents, such as is introduced by the use of milk in place of a part or all of the water in bread-making, has a marked influence on the nutritive value of bread, as may be seen in Fig. 15. The results are, however, progressively better with increasing proportions of milk in the diet up to at least one-third of the total solids of the food (Fig. 16).

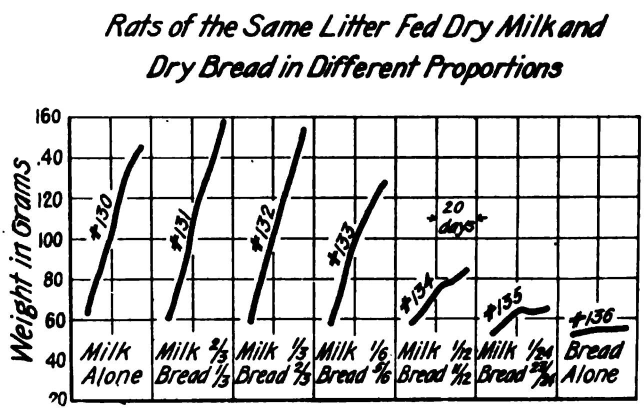


FIG. 16.—Growth curves of rats receiving different mixtures of dried whole milk and dried white bread. (By permission of the *Journal of Biological Chemistry*.)

With milk constituting one-third of the total solids of the food intake, almost equally good growth resulted whether the remainder of the diet were whole wheat or patent flour, and even when these were replaced by starch the result was not very strikingly different. But when the proportion of milk was decreased so that milk solids constituted one-sixth of the total dry weight of food, the superiority of whole wheat to patent flour and of patent flour to starch both became strikingly apparent. Compare the curves on the left with those on the right of Fig. 17.

The more a nation depends upon grain as food, the more important becomes the question of the best utilization of the germ of the grain and of the intermediate layers between the fibrous

seed coat and the starchy interior of the seed. Both the positive fear of digestive disturbance from the less highly refined mill products and the skepticism regarding the availability of their nutrients to man appear to be exaggerated. The repeated demonstrations of the fact that human beriberi may occur or be prevented depending upon whether a highly-milled or an undermilled rice is used as food certainly shows that the vitamin of the so-called coarser products is available to man.

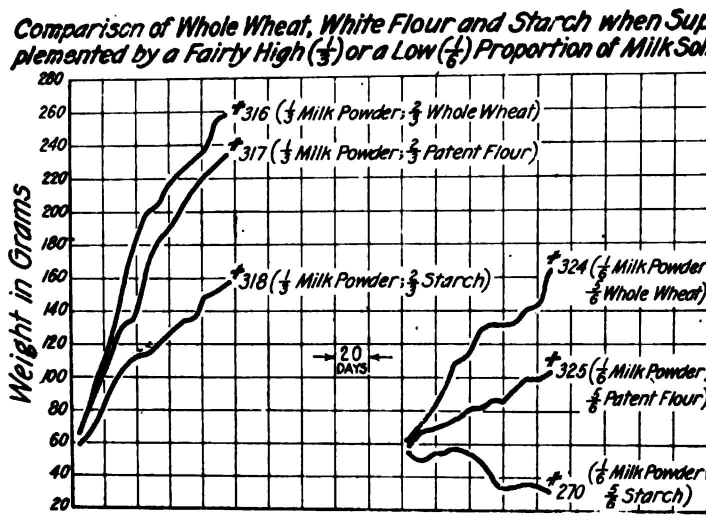


Fig. 17.—Showing that with a liberal proportion of milk in the food mixture the difference in nutritive value between whole wheat and patent flour appears small but that, as shown by a comparison at the right of the chart, as the grain product becomes more prominent in the diet the superiority of the whole wheat over the patent flour and of the patent flour over the starch becomes more marked.

The whole grain also shows its superiority when used in a diet for the support of reproduction and lactation in laboratory animals. In experiments recently reported, a mixture of five parts by weight of ground whole wheat and one part of whole milk powder supported normal growth and successful reproduction and rearing of young, while a corresponding mixture of patent flour and whole milk powder, while giving fairly comparable results in growth, was not adequate to the support of normal reproduction and successful suckling of the young (Sherman, Rouse, Allen and Woods, 1921).

The superiority of the whole grains over the products representing the endosperm only, is due to several factors.

Osborne and Mendel found the proteins of wheat embryo more efficient in nutrition, and those of the endosperm (patent flour) less efficient than those of the entire wheat grain. The proteins both of the bran and of the germ are quite efficient in supplementing the proteins of the endosperm. When the superiority of the mineral and vitamin contents of the germ and outer layers of the grain are also remembered it is plain that even though whole grain products are slightly less completely digested they are certainly far superior to the highly milled products in nutritive value if the latter term be used in as broad a sense as the newer knowledge of nutrition demands. Recent work also indicates that the difference in digestibility between properly prepared "whole wheat" bread and that made from patent flour is less than was formerly supposed. Probably for much the same reasons that they are more efficient in nutrition, whole wheat products when not properly kept are more susceptible to the ravages of insects and microorganisms than is patent flour, so that the latter can much more readily be kept for long periods without special care. This is a practical consideration of considerable weight but should not always be all-controlling, for the use of whole grain products need not result in large losses through spoilage if reasonable care be exercised in the handling.

It now appears that the difference in completeness of digestion is much more than compensated by the superiority of the whole wheat product in its mineral and vitamin content and in the nature of its proteins. In the amount of protein actually absorbed from the digestive tract, and in energy value, the difference between equal weights of whole wheat and patent flours is practically negligible. But a bushel of grain will make many more pounds of the actual, or even of the so-called, whole wheat flour than of patent flour so that even from the standpoint of protein and energy the best economy demands the milling of as high a percentage as seems practicable of the whole wheat kernel into human food, and from the vitamin standpoint this is also true and in much more striking degree. The parts of the grains now commonly rejected in milling them for human food are, of course, by no means entirely lost since their value in stock feeding is well known; but to the extent that it may be found wise to

increase the fraction of the wheat, rice or maize kernel devoted to human consumption there is no doubt that a true gain in efficient utilization of food resources will result because other and cheaper farm products which are not available for human food can be used in the feeding of animals.

Sugars and Fats.

Since the present work has to do chiefly with the vitamins, and since the relation of fat-soluble vitamin to good nutrition and health has been discussed in the preceding chapter, little need be said here regarding the sugars and fats. Sugar serves only as fuel and owes its popularity not to any nutritional advantage over other fuel foods, but simply to the fact that people like the sweet flavor. The greater the extent to which we depend upon refined sugar as food, the greater the danger that we may satisfy the appetite and the energy requirement without having fully covered all the other requirements of permanently adequate nutrition.

Fats also serve only as fuel in meeting the chemical requirements of nutrition, except in so far as butter and some other fats carry dissolved in the fat itself one of the necessary vitamins. In recent years butter has occupied a unique position because it appeared to be conspicuously the richest in vitamin A among the commercial food fats. Further work in this field indicates, however, that the value of butter in this respect is subject to rather wide variation. Steenbock, as previously noted, found that paleness of butter fat indicated paucity of vitamin A; but the present custom of artificially coloring butter makes it impossible for the consumer to apply this criterion. The burden of responsibility for the maintenance of the vitamin value of butter lies with the producer. By intelligent and liberal use of feeds rich in vitamin A (such as alfalfa), and care in the manufacture, transportation and storage of the product, both butter and other animal fats may in time be standardized as commercial sources of vitamin A. At present milk, eggs and green vegetables are probably more constant sources of vitamin A but fats enjoy favor because of other properties. Fat is a large factor in giving to food the quality of richness, so that shortage of fat for cookery is felt to be a hardship. Moreover, the larger the proportion of fat in the

food, the longer it is likely to stay in the stomach. Food very poor in fat tends to leave the stomach quickly, and this results in the early onset of hunger pangs. Thus the lack of fat is likely to be closely associated with the feeling of hunger and consequent lowering of morale and of working efficiency. It is largely for this reason that so much thought has been given to fat in the rationing of the nations at war. The Inter-Allied Scientific Food Commission considered it important to set standards for the total calories and for the fat of the food supply, though not for meat nor for protein.

Meats.

Meats are always rich either in protein or fat, often in both. A free use of meat in the diet contributes materially to the meeting of the energy and protein requirements. While one commonly thinks of the meats as chiefly significant for the protein which they contain, we now have reasons for believing that the popularity of meat as a food may be due as much to its flavor and its fat as to any benefit to be derived from its protein. Milk and eggs are even better sources of protein than meat and there is usually a much larger surplus of protein than of other nutrients in American dietaries; so it can hardly be because of a need for protein that meat is consumed in such quantity. Moreover, when the choice of food has been observed without any attempt to influence or control it, there has been found in American households a tendency for meat and fat to vary in inverse relation to each other in the food supply. Families buying less meat tend in general to buy more of the commercial fats and vice versa.

Meat is easily cooked and not easily spoiled in the cooking. It is easily digested even when hurriedly eaten, and its flavor is well liked by most people. These properties have doubtless played a large part in its popularity, backed as they are by the prevalent impression that meat-eating is in some special way related to strength and stamina.

But through the meat-saving campaign of the Food Administration, during the World War, many people have learned for the first time that it is usually beneficial to make milk more prominent than it hitherto has been in the average American dietary, even if this necessitates some decrease in the amount of

meat consumed. It is to be hoped that this shifting of emphasis from meats to dairy products in our diet will continue, as it will if the matter is fully understood by the consuming public, and must if our agricultural resources are to be utilized to the best advantage. This does not imply going without meat, but only being satisfied with a more moderate quantity than hitherto, and with beef chiefly grass-fed, as in most other countries, and not so largely grain-fed, now that the grain is needed for feeding people and milch cows.

On this point the National Research Council has recently issued the following statement prepared and unanimously adopted by its Committee on Food and Nutrition.

Meat and Milk in the Food Supply.

(COMMITTEE ON FOOD AND NUTRITION OF NATIONAL RESEARCH COUNCIL, APRIL 3, 1920.)

"It has long been known, but perhaps never sufficiently emphasized, that the milch cow returns in the human food which she yields, a very much larger share of the protein and energy of the feed she consumes than does the beef animal. Doctor Armsby (*Science*, August 17, 1917) has estimated that of the energy of grain there is recovered for human consumption about 18 per cent in milk, and only about 3.5 per cent in beef.

"In the report on the food supply of the United Kingdom, it was estimated that to produce 100 calories of human food in the form of milk from a good cow, required feed equivalent to 2.9 pounds starch; 100 calories milk from a poor cow was estimated to require the consumption of 4.7 pounds; and 100 calories of beef from a steer two and one half years old, to require 9 pounds starch equivalent in feed.

"Stated in terms comparable with those of Dr. Armsby this would mean that the good milk cow returns 20 per cent of the energy value of what she consumes; the poor milk cow 12 per cent; the good beef steer 6 per cent. Although this estimate is more favorable to the beef steer than is that of Dr. Armsby, yet even here it will be seen that the poor cow is twice as efficient, and the good milk cow more than three times as efficient as the beef steer in the conservation of energy in the food supply.

"Considering the whole length of life of the animal, Wood estimates that the cow returns in milk, veal and beef, 1/12 as much food as she has consumed, while the beef steer returns only 1/64; in other words, the cow is five times as efficient as the beef steer when the whole life cycle of the animal is considered.

"Similarly it has been estimated by Cooper and Spillman (Farmer's Bulletin, U. S. Department of Agriculture) that the crops grown on a given area may be expected to yield from four to five times as much protein and energy for human consumption when fed to dairy cows as when used for beef production. As Wood has very strikingly shown, the longer beef animals are fattened on grain, the less economical the process becomes.

"Quite recently Dr. Armsby has pointed out (*Yale Review*, January, 1920) that 'the dairy cow shows the highest efficiency of any domestic animal, both as regards conversion of food into milk and availability of the product for man.'

"Not only is the milk cow several times more efficient than the beef steer in the conservation of proteins, fats and carbohydrate for human consumption; in the gathering and preparation of mineral elements and vitamins she contrasts even more favorably with the beef animal. It is largely because of its richness in calcium and in fat-soluble vitamin that milk is the most efficient nutritional supplement to bread or other grain products.

"Meat is strikingly poor in calcium and does relatively little to balance a diet consisting largely of bread or of other products of seeds. It does, of course, supplement the protein, but American dietaries would nearly always be adequate as regards protein even without the meat that they contain. On the other hand, dietaries containing little or no milk are very apt to be inadequate as regards calcium. Detailed analysis of the results of hundreds of American dietary studies shows that in practice the adequacy of the calcium intake depends more largely on the adequacy of milk supply than upon any other factor, or in fact upon all other factors combined.

"The vitamins furnished by hay and grains and thus consumed by cattle are stored in the animal's tissues to only a limited extent, but are transferred in relative abundance to the milk. Hence, the vitamins of the coarse material not directly

available as human food, are brought into form for man's use very efficiently through milk production, and very inefficiently through the production of meat. Thus, the result of recent studies in nutrition which have made clearer the importance of the mineral elements and vitamins, is to emphasize strongly the great desirability of more abundant milk supply, even if this should somewhat reduce the production and consumption of meat. Our present knowledge of nutrition justifies more fully than ever before the statement that 'the dietary should be built around bread and milk,' bread or other grain products being the foods which furnish the most nutriment for their cost (whether in money or in land and labor) and milk being by far the most efficient nutritional supplement to bread or other grain products.

"Somewhat more of our grain crops than at the present should come directly into human consumption to augment the bread supply, and of the grain fed to cattle more should be used for the production of milk, and less for the production of meat.

"In general, 10 pounds of grain may be expected to produce not over 1 pound of meat or about 3 quarts of milk. If the 3 quarts of milk cost the consumer more (because of greater labor cost to produce) they are also certainly worth more to him. In so far as things so different as meat and milk in their nutritional properties can be compared, it is fair to say that one quart of milk is at least as great an asset in the family dietary as is a pound of meat. The per capita consumption of meat in the United States is so high that it might be reduced by one third or even one half with little or no nutritional loss, while a corresponding increase in milk consumption would certainly constitute a great improvement in the average American dietary.

"We are confident that a moderate shifting of emphasis from meat to milk will help in the normal evolution of American agriculture and improve the food economy and public health of the American people."

This statement of the National Research Council should not be construed as necessarily reflecting upon the prominence of meat in the American dietary but rather as primarily a plea for an adequate milk supply. America is fortunate in having such ample agricultural resources that our milk supply can be increased and our meat supply still continue fully adequate if not quite so high as in the past.

A moderate amount of meat often serves a very useful purpose in stimulating the appetite so that more food is consumed, and probably also promotes the secretion of gastric juice so that the food is more promptly digested in the stomach. But it should always be kept in mind that in order to get the full benefit from the meat it should not be taken in so large quantity as to displace too much of other food, but should rather be added in moderate amounts to a dietary in which the vitamins and mineral elements are already provided for by ample supplies of milk, vegetables and fruit.

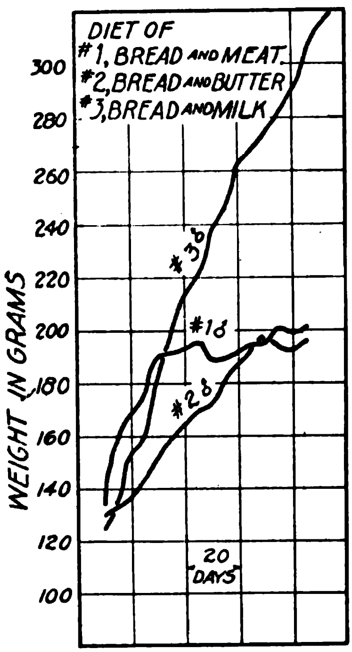


FIG. 18.—Growth curves of male rats of the same litter which when about one-third grown were placed upon diets of bread and meat, bread and butter and bread and milk respectively. See text.

It is especially to be remembered that as a group the meats show the same deficiencies in mineral elements and vitamins as do the grains, so meats are of little value in supplementing breadstuffs and other grain products in these important respects.

Fig. 18 shows growth curves of rats which when about one-third grown were placed on diets consisting respectively of bread and meat, bread and butter, and bread and milk. It will be seen that the diet of bread and meat resulted in better growth at first. In fact, for a length of time corresponding to more than a year in the life of a child, the animal receiving bread and meat appeared to the best advantage. Later, however, the deficiencies of the bread and meat diet manifested themselves first in a slackening and finally in a cessation of growth and the gradual development of a less vigorous condition. The animal receiving bread and butter grew slowly but steadily until finally he overtook the one receiving bread and meat and they both became moribund at about the same point. Bread and milk

resulted in steady good growth, always much superior to that on bread and butter, and overtaking and surpassing that on bread and meat in a time corresponding to about two years in the life of a child. We have here at least a strong hint and perhaps a fair explanation as to why meat and meat juice are usually given a more prominent place in children's dietaries in books written from the medical standpoint than in those written from the standpoint of food chemistry and normal nutrition. The early results of meat feeding are often very gratifying, appetite, digestion and body weight all showing improvement, and this improvement may easily continue as long as the child is under the care of the physician and perhaps much longer; but sooner or later if the meat is depended upon too largely its inferiority to milk in supplying the nutrients required for growth and development must become apparent to the student of the normal nutrition of the entire life cycle.

In experiments similar to those just discussed, but in which the comparison of bread and meat with bread and milk was begun at an earlier age, the failure of the meat to supplement the bread was more quickly apparent. Fig. 19 shows the results of feeding different rats of the same litter from weaning-time with bread alone or with bread and one other food.

In both the above mentioned experiments the bread supplied four-fifths, and the meat or milk supplied one-fifth, of the total calories of the food. When a part of the milk of such a bread and milk diet was replaced by meat the results were proportionately less disastrous than when meat replaced all the milk; but any replacement of milk by meat in such a dietary was always disadvantageous.

When, however, the dietary contains a liberal amount of milk so that all nutritive requirements are amply provided for, the addition of meat may result in nutritional advantage, at least in the support of reproduction and lactation. This is partly due, no doubt, to the fact that meat in moderation often improves the appetite and thus leads to the eating of a larger amount of the already good dietary. Whether the demand for extra protein in pregnancy and lactation, or any more specific relation of meat as a food to the nutritional processes involved in lactation is also a factor, remains to be determined.

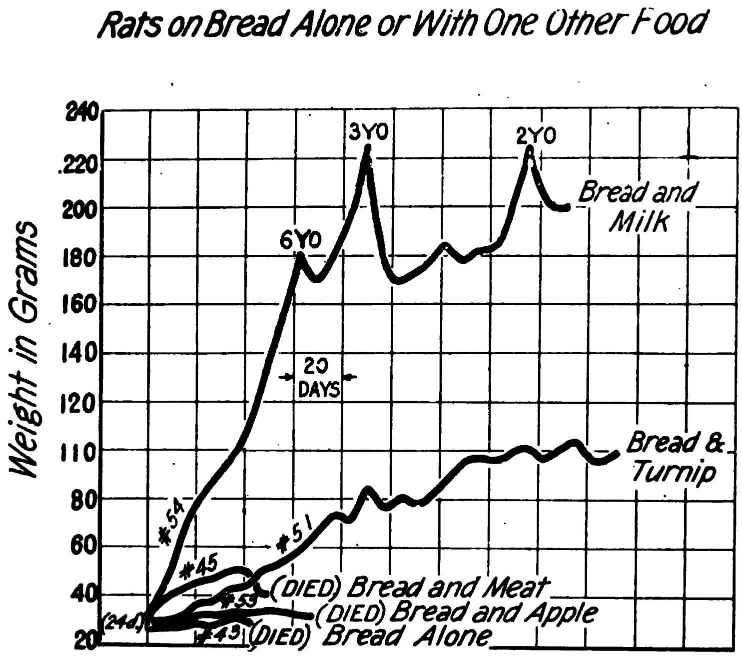


FIG. 19.—Growth curves of rats of the same litter placed at weaning time upon diets consisting of bread alone or bread and one other food. See text. (By permission of the *Journal of Biological Chemistry*.)

Milk.

Milk is the one article of diet whose sole function in nature is to serve as food and the one food for which there is no satisfactory substitute.

It is the sole food of most young mammals during their period of most rapid growth. The newborn rabbit doubles its weight in about six days, the lamb in fifteen, the calf in fifty, the colt in sixty, the baby in 180 days, and in each case normally upon a diet consisting exclusively of the milk of its mother. The milk of different species differs in quantitative composition with the rate of growth of the young, but the general characteristics of the milk of all mammals are the same. All milks provide all the substances needed for growth and when the milk of the mother fails, that of another individual or of another species is a much

better substitute than any other type of food and this for a number of reasons:

(1) The chemical structure or amino acid make-up of the milk proteins gives them exceptionally high nutritive efficiency as has been strikingly demonstrated in experiments upon both growth and maintenance by Osborne and Mendel and by McCollum, Simmonds and Parsons, and upon milk production by Hart and Humphrey.

(2) The fat of milk is of a low melting point as compared with most meat fats, and exists in an emulsified form, both of which properties are favorable to its ease and completeness of digestion. It also carries the fat-soluble vitamin which promotes growth and has such important functions in maintenance of health.

(3) The carbohydrate content of milk is in the form of milk sugar which is not only easy to digest but also has a more or less specific favorable influence upon the bacterial conditions in the digestive tract as has been recently emphasized by Rettger's extended research.

(4) Milk contains all of the inorganic elements or ash constituents required in human nutrition and furnishes them in exceptionally favorable proportions. In many experiments by Osborne and Mendel and by McCollum and his associates the growth and well-being of laboratory animals kept on restricted diets has been very greatly promoted by adding to the rations such salts as would give to the total inorganic content of the food mixture a composition corresponding to that of milk ash. Often indeed such improvement in the mineral content of the diet makes all the difference between complete success and total failure.

(5) Milk is exceptionally important as a source of vitamins. As a brief generalization, the statement of Rosenau that milk is rich in all three of the known vitamins may be accepted. If we would be more precise and critical we must take account of the differences between species not only as regards the quantitative composition of their milks and their relative nutritive requirements for different vitamins, but also as regards the effect of the vitamin content of the food and the stores of vitamins in the body upon the vitamin content of the milk produced.

Much emphasis is rightly given to the advocacy of maternal feeding but in order that breast milk may actually show the superiority that we are accustomed to expect of it, the attention paid to maternal feeding should take account of the food consumed by the mother as well as of the extent to which the mother nurses the child. In the mother's diet, milk should have a prominent place since it constitutes such an important supply of the nutrients from which the breast milk is to be formed.

Since the food of dairy cattle at all times of the year ordinarily contains an abundance of green leaves either fresh or preserved as silage, or dried as hay, there is thus ensured to the cow a liberal supply of both the A and B vitamins for transmission through her milk, and in the case of the A vitamin the dairy cow also carries a considerable store in her body so that the A vitamin content of cows' milk is probably in most cases fairly well maintained even though the food of the cow should for a time be deficient in this respect. It is of course possible to obtain, from cows fed for a long time upon feeds very poor in vitamin A, milk showing subnormal amounts of this vitamin, but such conditions are not frequent in good modern dairy farming, and cows so badly fed do not yield enough milk to constitute a large factor in the milk supply. There is probably a much larger seasonal variation in the amount of C vitamin contained in cows' milk than in either its A or B vitamin content. This being now recognized it is only necessary to use a small amount of orange juice or other antiscorbutic fruit or vegetable product in order with a liberal supply of milk in any of its various forms to ensure an ample intake of all three of the vitamins at all seasons.

The supplementing effect of the milk eaten with bread or other grain products is by no means exclusively a vitamin phenomenon. The richness of milk in vitamin A is undoubtedly very important in this connection but so is also the mineral content of the milk, and a further supplementary value lies in the fact that milk proteins supply abundantly certain amino acids (lysine, tryptophane and perhaps others) which the grain proteins as a whole contain in quantities too small to give them, when fed alone, a maximum efficiency in nutrition. In most cases of human malnutrition, as in the case of the smaller rat shown in Fig. 20, the fault of the diet may be traced in part to

FIG. 20.—Contrasting effects of equally simplified food supplies. These two rats were twin sisters and at weaning time were of equal size and equally healthy and vigorous. One was then fed with bread and apple, the other with bread and milk. The former remained stationary while the latter grew to five times the initial weight. The bread was identical in the two diets and the apple was of as good quality as the milk. It cannot be supposed that the apple had injured the rat inasmuch as this rat had survived others of the same litter which received bread alone or bread and meat as shown in Fig. 19. The difference in result was due to the superiority of the milk over the apple as a nutritional supplement of bread. In the case of the rat thus stunted by confining to a diet of bread and apple, as in most cases of human malnutrition, the fault of the diet was partly in its vitamin content and partly in other factors, conspicuously in this case a deficiency of calcium and phosphorus. (From Sherman, Rouse, Allen and Woods, 1921, by permission of the *Journal of Biological Chemistry.*)

its vitamin content and in part also to other factors among which deficiencies in calcium are perhaps the most frequent in American dietaries.

Cheese.

Cheese may be regarded as essentially a form of preserved milk, but differs necessarily in respect to those constituents removed in the whey. From our present standpoint this means that the water-soluble vitamins are less abundant, and the fat-soluble vitamin more abundant in cheese than in milk. Practically all of the fat, most of the protein, much of the mineral elements but only a small fraction of the milk sugar of the original milk is retained in the cheese. Regarded as a meat substitute, cheese is much richer in calcium and in vitamin A than are the ordinary meats.

Eggs.

In general terms eggs may be said to stand between meat and milk in nutritional characteristics. All three of these types of food are rich in proteins of a character efficient in supplementing the proteins of the grains; but as we have seen the mineral and vitamin contents of meat and milk are very different and in these respects the egg appears superior to meat and inferior to milk. As regards the two factors which McCollum regards as so often below the optimum in American dietaries, eggs rank with milk as a source of vitamin A but distinctly below milk as a source of calcium.

Nuts.

Because all are sometimes discussed as meat substitutes, it may be convenient to consider nuts next after cheese and eggs.

In chemical composition and biological significance the nuts as a group are essentially oil-bearing seeds, only a few of them being poor in fat and rich in starch. Like the other oil-bearing seeds, they show fairly high percentages of protein and such feeding experiments as have been made would seem to indicate that nut proteins rank high among the seed proteins in nutritional efficiency. As regards their mineral elements and vitamin values, the nuts in general resemble the whole grains.

Thus the typical nuts actually appear much like meat, both in their richness in protein and fat and in their lack of a satisfactorily balanced mineral and vitamin content. The nuts are, therefore, quite logical meat substitutes but cannot be regarded as substitutes for milk or as equivalents for cheese. Substitution of nut products for milk, cheese, butter or eggs is almost certain to mean a sacrifice in the fat-soluble vitamin value of the food. Nut products should be regarded as substitutes for other seed products or for meats and meat products, but not as satisfactory substitutes for dairy products or for eggs.

The commercial growing of nuts is an industry capable of great extension and can be developed with relatively little outlay of labor and to a considerable extent upon land of little value for ordinary farming.

Vegetables and Fruits.

Fruits and vegetables are important sources of the mineral elements and vitamins needed in human nutrition. Taken individually some are and some are not important sources of energy and protein as well. Potatoes, for instance, yield more calories of human food per acre than do wheat and rye. More labor is required to grow the potatoes, so they may be more or less economical than bread as sources of energy for the human machine according to the relative values of land and labor. Leaf vegetables, such as lettuce and spinach, are relatively unimportant as sources of energy, but are very effective supplements to the grains, being rich in the mineral elements and vitamins in which the grains are more or less deficient. Contrary to the supposition of former times, it now appears that a diet consisting largely of breadstuffs and cereals is more effectively supplemented by vegetables than by meat. This is true not only as regards the previously known factors of the food supply but also because of the richness in vitamins of the fruits and vegetables and because of their beneficial influence upon intestinal hygiene and upon the elimination of wastes from the body. A detailed analysis of the data of food supplies of over two hundred American families representative of a wide range of income groups and of various sections of the country, shows that on the average the money spent for fruit and vegetables is undoubtedly

better invested than the average of the money spent for food, so that the purchase of more fruit and vegetables will usually be good economy as well as good hygiene.

Increased attention to the marketing system and market news service of the nation is already helping both to advance the quality and to stabilize the supply of fresh fruits and vegetables. This will doubtless be further developed and with correspondingly increased benefit to the food supply of our cities. While only large-scale marketing methods can handle the bulk of the fruit and vegetable supplies of the large cities, yet there is also a place for increased production of vegetables in home gardens, both on farms and in towns and suburban districts. Neither the land nor the labor required for the village or suburban vegetable garden need be withdrawn from agriculture so that this, although confessedly a minor source of food supply, is capable of considerable development without in any way restricting the development of the major sources. The fact that garden vegetables are of value so largely because of their vitamins and that vitamin content is to some extent dependent upon freshness, makes it possible that something more than sentiment and gratification of the palate may be involved in our preference for vegetables "fresh from the garden."

In the average American food supply, vegetables represent about one-tenth of the expenditure for food and furnish about one-tenth of the total calories and of the total protein of the food; of calcium, phosphorus and iron they furnish much larger fractions and it is probable that the vegetables also furnish more than one-tenth of the vitamins in the average dietary.

Since the general dietary properties of the seeds and of the leaves have been much discussed already, attention will be given here chiefly to some typical vegetables of other types.

The ordinary potato, while superior to patent flour, ranks about with the whole grains in nutritive properties except that it has a considerable antiscorbutic property which in the grains can only be developed by careful sprouting.

Sweet potatoes, similar to ordinary potatoes in other respects, are, according to Steenbock, much superior to them and to the grains in their content of vitamin A, the sweet potato alone being adequate to furnish all that is needed of this vitamin, not only for growth but also for successful reproduction.

Carrots also were found by Steenbock to be rich sources of both the A and B vitamins.

Turnips are apparently less rich in vitamin A than carrots but may serve as an appreciable source of this vitamin as well as of calcium, as may be seen from Fig. 19, representing an experiment in which bread and turnips supported continued slow growth whereas bread alone or bread and meat early proved inadequate, probably because too poor in calcium and fat-soluble vitamin.

The rutabaga or Swedish turnip has been found to have high antiscorbutic value and its juice is used as a cheap and efficient substitute for orange juice in infant feeding. The larger use of these roots in the feeding of cattle should therefore improve the antiscorbutic vitamin content of their milk. Beets appear to be less rich in fat-soluble A than are carrots and less highly antiscorbutic than swedes (Swedish turnips) but Osborne and Mendel have shown that vitamin B occurs abundantly in the root, the stems and the leaves of the beet as well as in the bulb of the onion. Thus the cheaper winter vegetables can be relied upon to furnish material additions to the vitamin supply at times when fresh green vegetables are scarce or expensive.

Spinach, already prized for its mineral elements, is now found to be a rich source of all three vitamins as well. The same is true in only less degree of the other leaf vegetables.

Green string beans (edible seed pods) resemble leaves in dietary properties, ranking with cabbage in ability to support growth and reproduction in diets otherwise consisting too largely of white bread or flour with an amount of milk which was not in itself sufficient to make the food mixture adequate.

It has been seen that many of the green vegetables rank high as antiscorbutics and this property as well as their richness in vitamin A and in calcium is doubtless a factor in the important part which these vegetables play in the dietary of Oriental regions where milk supplies are even less adequate than in America and Europe. A former student, Professor Laird, of the Canton Christian College, writes that a typical working woman in that part of China eats $2\,^2/_3$ pounds rice, $1\,^1/_3$ pounds vegetables and a few cents worth salt fish or pork per day.

Another graduate student, Mr. Y. G. Chen, a native of the Nanking province, considers that green vegetables are probably

at least five times as prominent in the diet of that region as in the American diet. That children in the Orient fare as well as they do with a low per capita milk supply, is probably explained by the much longer time during which they receive their mothers' milk. With nursing continued often for two full years, and not rarely three, the child has ample time to become adjusted to the consumption of a variety of vegetable foods before its maternal milk supply is entirely cut off. It is also not improbable that the free use of green vegetables, with their high calcium and vitamin content, in the food of the mother may be a factor in her ability to continue normal lactation for such a long period. Certainly it would seem quite impossible for China and Japan to support such dense populations subsisting so largely upon rice and escape the deficiency diseases in so far as they do except for the large use that they make of the green vegetables and edible roots. Another factor of safety in the dietary habits of the Chinese is their use of sprouted seeds. In the Orient much use is also made of the young growing parts of even woody plants —bamboo sprouts, for example, being highly esteemed as food.

Tomatoes, formerly believed to be poisonous, and even within a decade stigmatized as little but colored water, are now regarded as one of the important staple foods because of their vitamin value. Special emphasis has been given to their richness in antiscorbutic vitamin which, doubtless because of the acidity of tomato juice, is in this case well retained through ordinary cooking, canning or even drying. Tomatoes have also been found to be rich in both the A and the B vitamins.

While ordinarily referred to as a vegetable the tomato is in reality a fruit and may serve to link the vegetables with the fruits in this discussion.

The importance of the fruits as sources of vitamins B and C has been discussed at some length in Chapters II and III and is to some extent summarized in the table given earlier in this chapter. Relatively little attention has yet been given to the examination of fruits for vitamin A so that an attempt to generalize on this point would probably be premature. Present knowledge of the value of fruits as sources of vitamins B and C supplies an economic justification for a fairly liberal use of fruit as food at prices which seemed high from the standpoint of our former, more limited knowledge of food values. With increased

efficiency of production and marketing, the development of more intensive methods of gardening, the extension of orchard areas particularly upon land too hilly to be used advantageously for crops requiring much cultivation, and with expanding facilities for trade with the tropics, there is no doubt that a larger supply of fruit will be provided as rapidly as consumers demand it.

To what extent the vitamin values of fruits will be influenced by storage, drying or canning is still a problem. The high vitamin value of canned tomato, the permanence of the antiscorbutic value of lemon juice as evidenced by its continued efficiency on long voyages, and the experiments upon drying and otherwise concentrating certain antiscorbutic fruit juices described in Chapter III are all facts which tend to give us hope that fruits may prove capable of preservation in various ways without too serious losses of vitamin values; but this is a subject too important to be left to inference. The values of different fruits as sources of each of the three vitamins and the influence of variety and size of fruit within the same species, of maturity when gathered, of storage at different temperatures and for varying lengths of time, of canning and drying under different conditions should all be investigated experimentally.

When research in this field brings us quantitative knowledge as to the efficiency with which vitamin C can be preserved in our staple fruits and vegetables when canned or dried, comparable with our present knowledge of the good preservation of vitamins A and B in cooked, canned or dried milk, it will become possible to plan food supplies, even for conditions in which fresh food cannot be obtained, with a similar degree of definiteness with reference to vitamins as to other nutritional needs.

Even with our present knowledge we believe it safe to say that with a dietary selected to make the best use of our ordinary staple foods there will rarely if ever be occasion to purchase vitamins in any other form, or to give any greater anxiety to the vitamins than to some other factors which enter into our present conception of nutritive requirements and food values.

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